

# Carcinogenicity of Saccharin

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Saccharin is carcinogenic for the urinary bladder in rats and mice, and most likely is carcinogenic in human beings. The neoplasms of the urinary bladder are malignant and invade and metastasize. Male rats are more susceptible to urinary bladder carcinogenesis than female rats. Rats exposed as fetuses develop neoplasms more readily than rats exposed as weanlings. The lesions in the urinary bladder go through the stages of hyperplasia, hyperplastic nodules, and later carcinomas. The male of the human species ingesting saccharin, as for rats, is more susceptible to carcinogenesis of the urinary bladder than the female. Neoplasms of the urinary bladder in rats were not caused by stones, parasites, sodium, or impurities. Carcinomas developed at the same time or prior to the development of stones in rats with both. There is a cocarcinogenic effect between saccharin and methylnitrosurea for the urinary bladder.

Even though carcinomas of the urinary bladder are present in rats given the higher doses of saccharin, one was observed in a female rat given 0.5%. Chronic renal disease develops in rats ingesting saccharin. The disease is more advanced at the lower doses than at the higher doses, suggesting that saccharin at the lower doses does not reach the urinary bladder. Early neoplasms are seen in the renal pelvis of rats given the higher doses of saccharin. The risk ratios for urinary bladder carcinomas in human beings increase with both frequency and duration of saccharin usage.

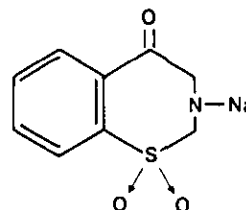
Benign and malignant neoplasms at all sites are significantly increased in mice and rats ingesting the higher doses of saccharin. These neoplasms are present in the reproductive and hematopoietic systems, and to a lesser extent in the lungs, vascular system and squamous epithelium. Neoplasms in some organs develop with the lower doses of saccharin. Lymphosarcomas of the lung are significantly increased in rats given 0.01% saccharin. Chronic renal disease in rats given saccharin interferes with the health and life span and consequently with the development of neoplasms. Saccharin initiates neoplasms of the skin when its application is followed by croton oil. Epidemiological studies have not been done for neoplasms other than the urinary bladder in human beings.

## Introduction

This report reviews the published and unpublished carcinogenicity studies on saccharin, a non-nutritive sweetener. They are from files at the National Academy of Science (1), Food and Drug Administration (2), and the National Cancer Institute (3). The results of only 5 of 11 studies in which rats ingested saccharin and 2 of 4 studies in which mice ingested saccharin have been published, and it is likely that most will never be published (Table 1). This review is my own analysis of the data which are, in most cases, raw data. Whenever possible, the number of animals is corrected for survival time.

The results are reported as tumors: in the urinary system, in the reproductive system, in the hematopoietic system, involving squamous epithelium, in the lungs and vascular system, and in all organs. In addition, studies in animals are compared to human studies.

The statistical analyses are one-sided *p* (probability) values (4).



SODIUM SACCHARIN

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Table 1. Studies reviewed.

Rat studies (oral)		
Lessel study (5-7)		
FDA study (1948-1949) (8)		
Schmähl study (9-10)		
Litton Bionetics study (12)		
Bio-Research Consultants study (14)		
Munroe et al. study (15)		
Hicks et al. study (1973, 1975) (17, 18)		
WARF Institute study (19)		
FDA study (1973) (20-22)		
Canadian Health Protection Branch study (1977) (23, 24)		
National Institute of Hygienic Sciences study (39)		
Mouse studies (oral)		
Bio-Research Consultants study (14)		
Kroes et al. study (30)		
National Institutes of Hygienic Sciences study (32)		
Roe et al. study (36)		
Other studies		
Althoff et al. study (38)		
McChesney et al. study (39)		
Allen et al. study (26)		
Bryan et al. study (27)		

## Tumors Involving the Urinary Bladder

### Rat Studies

**Lessel Study.** Boots-Wistar male and female rats, 20 per group, ingested 0%, 0.005%, or 5% saccharin in the diet (5, 6). Rats were killed after 2 yr. A positive control group was given subcutaneous trypan blue. Special attention was given to examination of the urinary bladder of a few rats, i.e., those that were grossly abnormal. Therefore, histological examination of the urinary bladder was inadequate.

"Abnormalities" of the urinary bladder occurred more often in the urinary bladder of males than in females, and were more frequent in rats ingesting 5% saccharin than in rats ingesting other doses (Table 2).

Histologic examination of the urinary bladder in one male rat ingesting 5% saccharin disclosed slight hyperplasia of the mucosa. One female rat ingesting 5% saccharin for 24 months had papillomatous

Table 2. Number of male and female rats ingesting saccharin with "gross abnormalities" of the urinary bladder.

Dose, %	No. of rats	
	Male	Female
0	2/20(10%)	0/20(0%)
0.005	1/20(5%)	0/20(0%)
0.05	4/20(20%)	0/20(0%)
0.5	1/20(10%)	0/20(0%)
5	5/19(26%)	3/18(17%)

<sup>a</sup> Rats surviving 6 months or longer.

hyperplasia with squamous metaplasia and another rat a papilloma of the bladder.

Four of five male rats with gross abnormalities had stones in the urinary bladder. Two of the bladders with stones were studied histologically, but apparently the bladder with a lesion and without stones was not studied. One female rat had a papilloma with a stone and another papillomatous hyperplasia without a stone. None of the rats given the lower doses or the controls had bladder stones.

The author states that there have been no lesions, tumors, stones, or nematodes of the urinary bladder occurring in untreated Boots-Wistar rats (7).

In this study, early tumors of the urinary bladder occurred in at least two female rats ingesting 5% saccharin. One tumor was associated with a bladder stone, but the other was not. The causal relationship of the tumors to the stones, or of the stones to the tumors, cannot be determined from this study. Animals need to be killed at frequent time intervals in order to study this problem.

**FDA Study (1948-1949).** E. L. Long and R. T. Haberman, pathologists at the FDA, with the assistance of Dr. Harold L. Stewart, reviewed all the available data and microscopic slides from the long-term rat feeding study on saccharin done at the FDA (8). Groups of 3-week-old Osborne-Mendel rats, 20 per group evenly divided by sex, were given 0%, 0.01%, 0.1%, 0.5%, 1%, or 5% saccharin in the diet for 2 yr. There was a control group of 53 rats. (It should be noted that there was only a slight degree of acute and chronic pneumonia in the untreated and treated rats of all groups.)

Although all urinary bladders were opened and described as being grossly normal, only one was sectioned and, on histological examination, appeared normal. Additional sections, at different levels, were cut from the original blocks of the kidneys with suspicious lesions.

Rats that had been given saccharin had papillary excrescences, calcification in the renal pelvis, and venous thrombosis, and the lesions were clearly dose-related (Table 3). The papillary lesions were present in 17% of rats at the 1% dose level, and in 76% of the rats at the 5% dose level of saccharin. There were calcifications in 76% and venous thrombosis in 24% of rats given 5% saccharin. There was also chronic interstitial nephritis that was much more severe in rats given saccharin, but was not related to the papillary lesions of the renal pelvis. No sex differences were evident.

The lesion "was characterized by small papillary projections or excrescences from the renal papilla, calyx, or papillo-calyceal junction. . . . They appeared to be the result of a combination of the following changes: very slight to marked degrees of

**Table 3. Lesions of the renal pelvis in male and female rats ingesting saccharin.**

Dose, %	No. of rats		
	Epithelial Hyperplasia	Calcification	Venous thrombosis
0	1/33(2%)	1/53(2%)	0/53(0%)
0.01	0/13(0%)	0/13(0%)	0/3 (0%)
0.1	0/15(0%)	0/15(0%)	0/15(0%)
0.5	1/15(7%)	2/15(13%)	0/15(0%)
1	3/18(17%)	1/18(6%)	0/18(0%)
5	13/17(76%) <sup>a</sup>	13/17(76%)	4/17(24%)
	( $p = 0.000000084$ ) <sup>b</sup>	( $p = 0.000000001$ ) <sup>b</sup>	( $p = 0.000742$ ) <sup>b</sup>

<sup>a</sup> The additional sections increased the number of lesions in the rats given 5% saccharin from 5 to 13.

<sup>b</sup> Statistical analyses are one-sided  $p$  (probability) values (4).

stratification of the epithelium and the single layer of cuboidal cells that normally cover the papilla and calyx of the rat to several layers of cells (i.e., to the transitional type which in the rat is normal in the renal pelvis but not in the calyx or papilla) in all affected animals."

They concluded that "this lesion of the renal papilla was undoubtedly the result of the administration of saccharin." They went on to state "the question naturally arises as to the relation of these renal lesions to the papillary carcinomas and papillomas of the bladder produced by the recent Abbott study employing a mixture of cyclamates and saccharin, since the papilla and calyx are adjacent to the pelvis and both bladder and renal pelvis are lined by similar epithelium (transitional) and would consequently be expected to give rise to similar tumors."

With regard to the failure to section the urinary bladders histologically: "In view of the Abbott cyclamate-saccharin study in which, although some of the bladder tumors found were visible grossly, others were identified only after histologic examination. . . . While there was epithelial hyperplasia in the FDA rats, in no case was there a true tumor, although in one case (10078) the proliferation was striking enough to be called papillomatoid or preneoplastic," and implied that this study could not be considered a negative one for tumors of the urinary bladder.

The authors said with reference to the renal pelvis lesion. "It is also a lesion we have never seen before." The lesion occasionally occurs in rats ingesting a renal and hepatic carcinogen, *N*-4-(4'-fluorobiphenyl) acetamide, in the diet, as well as in the kidney of rats given several chlorinated hydrocarbon pesticides (9). In some cases, the lesions were advanced enough to be reported at least as papillomas or preneoplastic lesions. The epithelial hyperplasia in the renal pelvis is identical to that considered as preneoplastic or a precursor to trans-

itional carcinomas of the urinary bladder, and should therefore be considered as a precursor of transitional cell carcinoma in the renal pelvis. In most instances, however, the lesion was discovered on random sectioning of the kidney, and not after a careful systematic examination.

The presence of a dose-related response to the development of epithelial hyperplasia of the renal pelvis suggests that the earliest effects of saccharin are on the epithelium of the renal pelvis. There was also treatment-related chronic renal disease in rats. It may well be that higher dose levels of saccharin are necessary in order for sufficient levels to reach the urinary bladder and to induce lesions there.

In summary, saccharin given in the diet to both male and female Osborne-Mendel rats induces transitional cell hyperplasia of the renal pelvises and calyces, referred to as papillary lesions. The lesion is dose related, with the most severe lesions occurring at the 5% dose level. Other chemicals cause a similar more advanced preneoplastic hyperplasia or papillomas in the renal pelvis.

The following conclusions were drawn. Since the urinary bladders were not sectioned and studied histologically, this study cannot be considered as negative for tumorigenicity of the urinary bladder. Because of the similarity of the epithelium of the renal pelvis and the urinary bladder, there exists the potential for the induction of similar lesions in the urinary bladder. The epithelial hyperplasia in the renal pelvis is identical to that in the urinary bladder that is considered to be a precursor to carcinomas of the urinary bladder, and should therefore be considered as a precursor to carcinoma of the renal pelvis. It may be necessary to administer higher doses of saccharin in order for sufficient levels to reach the urinary bladder after the earlier contact with the kidney and renal pelvis.

The results of this study suggest that more complete examinations of the urinary system are necessary in rats given saccharin. The epithelium of the renal pelvis and ureters, as well as the interstium of the kidneys, should also be studied in addition to the urinary bladder.

**Schmähl Study.** Schmähl studied the effect of saccharin, with emphasis on the urinary bladder, in Sprague-Dawley rats because they developed carcinoma of the urinary bladder when given butyl-butanol nitrosamine (10, 11). Fifty-two male and 52 female rats, 70-90 days old, were given 0%, 0.2%, or 0.5% sodium saccharin in a pellet diet for their lifetime. They also gave rats the much higher doses of 2% or 5% cyclamate.

The urinary bladder was examined histologically in all rats; however, further histologic examinations were done "when organs seemed pathologically

changed." All rats started in the study were included as at risk in the study. The results were not separated by sex.

There were no carcinomas of the urinary bladder in rats ingesting saccharin. One rat given 2% cyclamate for 27 months developed a transitional cell carcinoma of the urinary bladder, which was attributed to bladder stones. The 16% of rats with parasites (*strongyloides*) observed on histologic sections of the urinary bladder, did not have lesions or tumors of the bladder.

It was concluded that at the relatively low dose levels of 0.2% or 0.5%, carcinomas were not observed in the urinary bladders of rats.

The results obtained were under the conditions of this experiment: incomplete histology, failure to separate the results by sex, including all rats at risk for the development of tumors, and using a strain with a high incidence of "spontaneous" tumors. Furthermore, "most of the animals died from typical infections of the respiratory tract."

Rats with urinary bladder parasites were described as not being more susceptible to the induction of bladder tumors.

Emphasis appears to be placed on the development of tumors, without describing other changes.

**Litton Bionetics Study.** Litton Bionetics studied the chronic effects of sodium saccharin in Charles River CD strain male and female rats (12). Rats ingested 0%, 1%, or 5% sodium saccharin in the diet for up to 2 yr. There were two duplicate studies with the same experimental design.

One of 26 female rats ingesting 5% sodium saccharin had a papilloma of the urinary bladder in the second study. One of 26 male rats ingesting the high dose developed hyperplasia of the urinary bladder in the first study. Lesions of the urinary bladder were not found in groups of 20 male and 20 female controls in either study.

Chronic glomerulonephritis was found in 17 of 26 (65%) males and 1 of 26 (4%) females ingesting 1% saccharin in the diet. No mention was made of lesions of the renal pelvis.\*

The data from these studies was analyzed at the NCI and the following conclusion was drawn (13). "A papilloma of the urinary bladder was found in a single female of the high dose saccharin group in the second replicate. This was the only bladder tumor found in the study. In the first replicate, hyperplasia of the urinary bladder was found in the high dose male group. Although these findings are not statistically significant, they do not, on the other hand, show saccharin to be safe. Because of the small

\* In view of the renal pelvic lesions described in the kidney by Long and Haberman, it would be desirable to reevaluate the kidney sections in this study also.

numbers of animals tested, this experimental result is consistent with very high possible urinary bladder tumor effect by saccharin in rats."

The conclusion that can be made from this study is that it is likely that saccharin is tumorigenic for the urinary bladder. Saccharin at the 1% dose level also induced a high incidence of chronic renal disease in male rats.

**Bio-Research Consultants.** Bio-Research Consultants carried out two separate studies with the same experimental design on the chronic effects of sodium saccharin in rats (14). The saccharin from two sources (Merck or Monsanto) was stated as not containing impurities. Charles River Sprague-Dawley male rats, 25 in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. Female rats were not included in this study.

Millipore filter preparations were made of urine of male rats at the time of autopsy, or, if the bladder was empty, of saline washings of the bladder. The filters were stained and studied for parasites and ova. The bladder was then inflated with formaldehyde, the urethra ligated, and the bladder fixed. Histological sections of bladders and kidneys were reviewed by G. W. Friedell, St. Vincent Hospital, Worcester, Massachusetts. Urinary bladders were not examined if rats were too autolyzed for autopsy.

There was one carcinoma of the urinary bladder in a rat on the high dose of saccharin and one papilloma in a rat on the low dose in one group (Table 4). In the other group, one carcinoma was present in a rat on the low dose and one papilloma was found in a rat on the high dose. One control rat also had a carcinoma of the urinary bladder.

Ova consistent with *Trichosomoides crassicauda* were present in urine of approximately one-third of the rats. There was no correlation between the parasite and tumors of the bladder.

The transitional cell epithelium of the renal pelvis frequently had atypical hyperplasia, often associated with gross calcification in rats of all groups. However, this lesion was seen most often in male rats ingesting the high dose of sodium saccharin. One rat ingesting the low dose of saccharin had an "adenoma" of the kidney.

The incidence of tumors of the urinary bladder

Table 4. Tumors of the urinary bladder in male rats ingesting sodium saccharin in both studies.

Dose, %		No. rats		
		Group 1	Group 2	Total
0	Matched			1/16(6%)
	Pooled			5/111(5%)
1		1/13(8%)	1/15(7%)	2/28(7%)
5		1/12(8%)	1/14(7%)	2/26(7%)

was not increased in saccharin-treated rats; however, rats with papillomas or carcinomas of the urinary bladder did not have bladder stones and there was no relationship to parasites of the urinary bladder. The saccharin was stated as not containing impurities.

Tumors of the renal pelvis (atypical hyperplasia) often seen in male rats ingesting the high dose of saccharin have been described in other previous chronic feeding studies in rats (8).

**Study of Munroe et al.** Munroe et al. studied the carcinogenicity of commercial saccharin in male and female Charles River weanling rats weighing 50–60 g (15). Groups of 60 male and 60 female rats were given sodium saccharin at levels of 0, 90, 270, 810, or 2430 mg/kg-day in a synthetic diet for 26 months. Constant levels were given by adjusting amounts at weekly intervals. The urinary bladder, as well as "other grossly abnormal organs," were examined histologically. At the start, 10 rats per group, and at 23 months all surviving rats, were examined for the bladder parasite *T. crassicauda* by collecting and examining samples of urine for parasite eggs.

Four transitional cell papillomas of the urinary bladder were observed in the treated rats and none in the control rats (Table 5). The only other lesion of the epithelium was malakoplakia in one male receiving the highest dose level of sodium saccharin. Two of the papillomas were described as 2 cm in size and entirely filled the bladder cavity; the other two did not exceed 3 mm. Three rats with urinary bladder calculi did not have tumors of that organ. Ova of *T. crassicauda* were not found in the urine.

Histological sections of the bladder tumors were reviewed by a NAS Subcommittee Panel on Pathology in 1973. There was unanimous agreement that two of the tumors diagnosed as papillomas were transitional cell carcinomas, one with invasion (16).

There is an illustration of a "calculus" in the

medulla of the kidney of a saccharin-treated rat; however, data as to numbers of rats with such histologic lesions is unreliable if only selected kidneys are sectioned and studied histologically (8).

It was concluded that small numbers of sodium saccharin-treated rats, under the conditions of this study, developed transitional cell papillomas and carcinomas of the urinary bladder. The incidence is not certain because the number of effective rats was not given.

The rats with tumors of the urinary bladder were free of the bladder parasite *T. crassicauda* and did not have bladder stones. Parasites and bladder stones, therefore, were not the cause of the bladder tumors.

It cannot be concluded from this study that saccharin is not carcinogenic for the urinary bladder. There were, in fact, two transitional cell papillomas and two transitional cell carcinomas (one invasive) of the urinary bladder in the treated rats suggesting that saccharin is most likely carcinogenic for the urinary bladder.

**Study of Hicks et al. (1973).** A rat model for studying the induction of urinary bladder cancer using intravesicular instillation of methylnitrosourea (MNU) was developed by Hicks et al. (17). A single dose of 2.0 mg MNU into the bladder of a 150 g Wistar rat acts as an initiator but does not produce carcinoma unless its action is promoted by further doses. In the first few weeks there is a transient hyperplastic response of the bladder epithelium; however, the epithelium is normal in appearance after more than 12 weeks.

The effect of dietary saccharin on the response of the urinary bladder to MNU was studied in female Wistar rats, 50 per group, ingesting 41B pelleted diet. In the first group, each rat had a single dose of 2 mg MNU instilled through urethral catheter into the bladder at the start of the study. In the second group, saccharin was added to the drinking water; the rats were weighed and the saccharin content of the water adjusted to maintain an intake of 2 g/kg of body weight daily throughout the study. Rats in the third group received saccharin and each rat had a single dose of MNU 6 weeks after the start of the saccharin regime. Control rats received no treatment. Twelve rats in each group were killed between 3 and 56 weeks. The urinary bladders were distended with fixative at necropsy and examined grossly and histologically.

Nine of 12 rats (75%) receiving both MNU and saccharin developed marked changes of the urinary bladder epithelium (Table 6). Three (25%) has invasive transitional and squamous cell carcinoma. In two others there were hyperplastic nodules and papillomas. Some animals also had calculi in the

**Table 5. Male and female rats ingesting saccharin with epithelial tumors of the urinary bladder.<sup>a,b</sup>**

Dose, mg/kg	No. rats		
	Males	Females	Total
0	0/57 <sup>c</sup>	0/56	0/113
90	1/51	1/56	2/107
270	0/59	0/52	0/106
810	2/52	0/56	2/108
2430	0/54	0/54	0/108

<sup>a</sup> Total number is equal to the number of rats examined histologically.

<sup>b</sup> The effective number of rats, or rats that survived long enough to develop tumors, is not given. Only about 40 rats per group were alive at 18 months.

<sup>c</sup> One rat in this group had an "angiosarcoma" of the submucosa which was of vascular origin.

**Table 6. Lesions of the urinary bladder in rats receiving saccharin and methylnitrosourea (MNU).**

Lesion	No. of rats	Time killed, weeks	No. of rats with calculi <sup>a</sup>
None	1	20	0
Mild hyperplasia	2	3, 10	1
Severe hyperplasia	4	12, 13, 16, 30	1
Hyperplastic nodules and papillomas	2	8, 24	2
Carcinomas with invasion	3	21, 50, 50	3

<sup>a</sup> Of rats given MNU, 20% develop only calculi without tumors.

bladder. Two of 12 rats given saccharin only had mild hyperplasia after 36 weeks, and none had calculi.

The results indicate that rats given both MNU and saccharin develop varying degrees of hyperplasia, followed by hyperplastic nodules and carcinomas with invasion. Severe hyperplastic lesions were present after 12 weeks, nodules after 24 weeks, and carcinomas after 50 weeks. Rats given MNU only had transient mild hyperplasia. Therefore, it is clear that saccharin was responsible for the progression of that mild hyperplasia to more advanced tumors and carcinomas. Rats given MNU plus cyclophosphamide developed hyperplasia but not carcinomas of the urinary bladder. Hyperplasia that is not severe and that does not develop early will not become nodules and carcinomas.

Urinary bladder calculi were present in 7 of 12 rats given saccharin and MNU; whereas 20% of rats given MNU only develop calculi without tumors. Calculi developed after tumors of the urinary bladder and were not responsible for the tumors in rats given both chemicals.

Remaining animals were to have been killed during the second year of the study; however, the results have not been obtained.

The authors concluded: "Our experiments show that saccharin is cocarcinogenic in conjunction with a single dose of MNU, and indicate that a promoting action of saccharin with cyclamate and/or other bladder carcinogens is an obvious possibility." They also pointed out that cyclamate and saccharin was usually consumed together by man in a 10:1 ratio mixture.

**Study of Hicks et al. (1975).** Wistar male and female rats were given 2 or 4 g/kg of body weight daily of sodium saccharin for 2 yr (18). Other groups of female rats were given a single dose of 1.5 mg of MNU into the urinary bladder. Other rats received cyclamate or cyclophosphamide.

A small number of rats given 4 g/kg sodium saccharin alone developed tumors of the urinary bladder (3 of 138) after 95 weeks. Twenty-three of 49

(47%) and 27 of 47 (57%) MNU-treated rats receiving saccharin had tumors of the urinary bladder as early as 10 weeks (Table 7). The tumors in MNU-treated rats were more malignant and invasive than those in rats given saccharin alone. Urinary bladder tumors were not seen in rats given MNU alone. Bladder tumors had not been observed "spontaneously" in 600 rats in over a 10-yr period.

Some of the rats with tumors of the urinary bladder also had stones. Thirteen rats given a single dose of MNU had stones, but none developed tumors. Five rats receiving MNU plus saccharin and four given MNU plus cyclamate had bladder tumors without stones. The authors concluded: "In these experiments a calculus alone was thus not able to promote tumor formation in an MNU-primed bladder in the absence of some further specific carcinogenic stimulus. That further stimulus was clearly given by the additional doses of MNU or by including saccharin or cyclamate in the diet."

"The calculus is thus not an obligatory factor in the syncarcinogenic effect of these sweeteners. For all this work specific pathogen-free rats were used and there were no infections with the worm, *Trichosomoides crassicauda*."

They also discussed the use of an animal model, which depends upon the synergy of compounds, for the testing of chemicals for carcinogenicity.

"Man, however, is exposed to a complex environment, in which he may simultaneously encounter potentially carcinogenic chemicals in the food he eats, in the air he breathes, in the tobacco smoke he inhales, and in the factory where he works. Any individual suspect compound may be present in such low amounts that if tested singly at 'relevant' concentrations its effect may be negligible or even undetectable, even though in combination with other environmental factors its effect could well be significant. There is thus some justification for looking for compounds which are synergistic with other known bladder carcinogens."

In summary, this study demonstrated a cocarcinogenic effect between methylnitrosourea and saccharin. Stones and parasites were not responsible for this carcinogenicity.

**Table 7. Tumors of the urinary bladder in rats given methylnitrosourea and/or saccharin.**

Dose, g/kg-day	No. of rats with tumors of urinary bladder	
	Saccharin	MNU + saccharin
2	0/115(0%) <sup>a</sup>	23/49(47%) ( $p = 0.000000000000008709$ )
4	3/138(2%) <sup>a</sup>	27/47(57%) ( $p = 0.00000000000000132$ )

<sup>a</sup> Male and female rats were combined.

**Wisconsin Alumni Research Foundation (WARF) Institute Study.** The WARF Institute (International Sugar Foundation) study used Sprague-Dawley ( $F_1$ ) male and female weanling rats (19). ( $F_1$  were the litters of female rats given saccharin. Treated rats were exposed to saccharin from inception.) Male and female rats, 20 per group, ingested 0%, 0.05%, 0.5%, or 5% sodium saccharin in Purina laboratory chow for 100 weeks. Most tissues were sectioned histologically, including the urinary bladder.

Male rats, particularly at the 5% dose level of saccharin, developed a high incidence of bladder tumors (50%), and most of the tumors were transitional cell carcinomas; whereas the untreated males did not have a single tumor of the urinary bladder ( $p = 0.00123$ ) (Table 8). One female rat given 0.05% saccharin had an undifferentiated malignancy in the urinary bladder (Table 9). Stones were not present in the urinary bladder.

Histological sections of some of the bladder tumors were reviewed by a Subcommittee Panel on Pathology in 1973. There was unanimous agreement that the tumors were transitional cell carcinomas and that two of the carcinomas were invasive (16).

Additional data, including lesions of the renal pelvis and kidneys, would be helpful in interpreting the relationship of dose to lesions of the urinary bladder. Information concerning parasites was not given.

In conclusion, saccharin is carcinogenic for the urinary bladder of rats. Some of the carcinomas were invasive. Stones were not related to the development of urinary bladder tumors. Male rats were more susceptible to the development of carcinomas of the urinary bladder than were female rats.

**FDA Study (1973).** Male and female Charles River Sprague-Dawley rats,  $F_1$  generation, 48 per group, ingested 0%, 0.01%, 0.1%, 1%, 5%, and 7.5% sodium saccharin in the diet for 28 months (20). Control rats were given a diet containing sodium carbonate at the same level as that ingested by the rats given 5% sodium saccharin. Some rats were killed after 14 and 18 months. Rats from the  $F_0$  generation were killed after 4, 6, and 12 months (21). Special attention was given to examining the urinary bladder.

The incidence of carcinomas and papillomas of the urinary bladder were significantly increased in male rats ingesting 7.5% saccharin for 24 months ( $p = 0.01755$ ) (Table 10). The incidence of carcinomas and polypoid hyperplasia of the urinary bladder was increased in female rats given the same dose level (Table 11). Males were more susceptible to carcinogenesis of the urinary bladder than female rats.

**Table 8. Lesions of the urinary bladder in male rats ingesting saccharin.**

Dose, %	No. of lesions		
	Hyperplasia of the urinary bladder	Carcinoma of the urinary bladder	Total tumors
0	0/16(0%)	0/16(0%)	0/16(0%)
0.05	0/16(0%)	0/16(0%)	0/16(0%)
0.5	1/15(7%) <sup>a</sup>	0/15(0%)	1/15(7%)
5	1/16(6%) <sup>b</sup>	7/16(44%)	8/16(50%)
		( $p = 0.00340$ )	( $p = 0.00123$ )
		( $p = 0.0000185$ ) <sup>c</sup>	( $p = 0.000026$ ) <sup>d</sup>

<sup>a</sup> Considered as "precancerous."

<sup>b</sup> Described as "papillary projections."

<sup>c</sup> Tested against 0/48.

<sup>d</sup> Tested against 1/47.

**Table 9. Lesions of the urinary bladder in female rats ingesting saccharin.**

Dose, %	Carcinoma of the urinary bladder
0	0/17(0%)
0.05	1/17(6%) <sup>a</sup>
0.5	0/15(0%)
5	0/20(0%)

<sup>a</sup> Undifferentiated malignancy.

**Table 10. Lesions of the urinary bladder in male rats ingesting sodium saccharin in the diet for 24–28 months.**

Dose %	No. of lesions		
	Papillomas of the urinary bladder	Carcinomas of the urinary bladder	Total tumors of the urinary bladder
0	0/25(0%)	1/25(4%)	1/25(4%)
5	0/21(0%)	1/21(5%)	1/21(5%)
7.5	1/23(4%)	6/23(26%)0.038	7/23(30%)0.01755
		( $p = 0.013903$ ) <sup>a</sup>	( $p = 0.00489$ ) <sup>a</sup>

<sup>a</sup> Tested against 2/46.

**Table 11. Lesions of the urinary bladder in female rats ingesting sodium saccharin in the diet for 24 months.**

Dose, %	No. of lesions		
	Polypoid hyperplasia of the urinary bladder	Carcinomas of the urinary bladder	Total tumors of the urinary bladder
0	0/24(0%)	0/24(0%)	0/24(0%)
5	0/28(0%)	0/28(0%)	0/28(0%)
7.5	2/31(6%)	2/31(6%)	4/31(13%)
			( $p = 0.09226$ )
			( $p = 0.01712$ ) <sup>a</sup>

<sup>a</sup> Tested against 0/52.

The Subcommittee of Pathology reviewed selected slides from this study and confirmed the presence of transitional cell carcinomas, one with invasion of the urinary bladder (16).

Lesions of the urinary bladder were classified ac-

cording to the severity of the hyperplasia, i.e., minimal isolated focus of hyperplasia of the mucosal epithelium, slight hyperplasia of the mucosal epithelium, moderate hyperplasia of the mucosal epithelium, and marked hyperplasia of the mucosal epithelium. The tumors were classified as polypoid hyperplasia of the mucosal epithelium, transitional cell papillomas, early transitional cell carcinomas, and transitional cell carcinomas (usually papillary).

The severity of the hyperplasia and the development of tumors of the urinary bladder were related to the duration of ingestion of sodium saccharin, as well as to the dose level ingested. At the end of 4 months animals at the lower doses had minimal isolated foci of hyperplasia, except for two rats given 7.5% saccharin that had slight hyperplasia. Rats developed slight and moderate hyperplasia by 6 months and moderate hyperplasia by 12 and 18 months.

Polypoid hyperplasia, papillomas, and transitional cell carcinomas were present in rats at 24 to 28 months given the 7.5% dose level. One male rat given 5% saccharin had an early transitional cell carcinoma. Rats with polypoid hyperplasia, papillomas, or carcinomas sometimes also had moderate or marked hyperplasia. In addition, rats without tumors at the lower dose levels had hyperplasia of the urinary bladder at 24 to 28 months.

Hyperplasia of the urinary bladder was increased in male rats (32%) and in female rats (15%) given 7.5% sodium saccharin (Tables 12 and 13). Control male rats with bladder lesions had slight or moderate hyperplasia and female rats had slight hyperplasia at 24 to 28 months. The increase in hyperplasia is significant in the male and female rats ingesting 7.5% saccharin ( $p = 0.004046$ ).

There were other changes observed in the urinary bladder; however, these lesions were not related to the development of hyperplasia or tumors. These were mucus, proteinaceous casts, and cystitis. A few rats with mucus and proteinaceous casts also had hyperplasia of the urinary bladder; however, most did not. Five rats with slight or moderate hyperplasia also had cystitis.

Perhaps of greater interest was the finding of bladder stones and the absence of parasites (Table 14). Bladder stones were found in 67% of treated male rats at the end of 12 months. Not one rat with bladder stones developed either hyperplasia or tumors of the urinary bladder. Bladder stones were not observed in female rats. Parasites of the urinary bladder were not observed.

Chronic interstitial nephritis was more advanced at the lower dose levels and less advanced at the higher dose levels of sodium saccharin; whereas "polyposis" of the calyceal of the kidney was the

**Table 12. Number of male rats ingesting saccharin at various dose levels with hyperplasia of the urinary bladder.<sup>a</sup>**

Duration, months	No. of rats					
	0	0.01%	0.1%	1%	5%	7.5%
4	0/15	0/16	1/19	1/19	1/7	2/4
6	1/29	1/31	0/35	0/32	3/20	4/18
12	1/12	1/18	0/15	1/15	0/16	4/15
18	0/7	1/6	0/4	0/7	0/7	4/6
24	8/24	3/16	4/27	3/22	3/21	7/23
Total	10/87	6/87	5/100	4/95	7/61	21/66
	(12%)	(7%)	(5%)	(4%)	(11%)	(32%)

( $p = 0.00190$ )

<sup>a</sup> Rats with tumors that also have hyperplasia are included.

**Table 13. Number of female rats ingesting saccharin at various dose levels with hyperplasia of the urinary bladder.<sup>a</sup>**

Duration, months	No. of rats					
	0	0.01%	0.1%	1%	5%	7.5%
4	0/13	0/13	0/18	1/17	0/8	1/4
6	1/48	0/49	0/48	0/50	1/37	4/35
12	0/7	0/5	0/3	0/4	1/6	0/5
18	0/6	0/4	0/5	0/6	0/7	0/5
24	2/24	0/23	0/24	3/30	3/28	7/31
Total	3/98	0/94	0/98	4/107	5/86	12/80
	(3%)	(0%)	(0%)	(4%)	(6%)	(15%)

( $p = 0.004046$ )

<sup>a</sup> Rats with tumors that also had hyperplasia are included.

**Table 14. Number of male and female rats ingesting sodium saccharin with bladder stones.**

Duration, months	No. bladder stones			
	Male rats		Female rats	
	Treated	Untreated	Treated	Untreated
4	0/70	0/15	0/65	0/13
6	6/209	0/48	0/176	0/29
12	12/18	2/7	0/58	0/12
18	1/28	0/6	0/30	0/7
24	0/136	0/24	0/109	0/25
Total	19/461	2/90	0/438	0/86

opposite. Polyposis, which was not described in detail, occurred more frequently in the rats given 7.5% saccharin than in the controls ( $p = <0.05$ ) (22).

Male rats with carcinomas of the urinary bladder ingesting higher dose levels of sodium saccharin had more severe polyposis of the calyceal of the kidney, and less advanced chronic nephritis. Hyperplasia of the urinary bladder developed as early as 4 months, but polyposis was not seen until 18 months. This finding suggests that if polyposis is present in male rats after 18 months, it is associated with lesions of the urinary bladder.



The FDA Study (1948-1949), as analyzed by Long and Haberman with the help of Stewart, suggests that this study of urinary bladder is not complete without a careful examination of the epithelium of the renal pelvis, as well as the entire kidney and ureter (8).

Conclusions that can be drawn from this study are as follows.

Sodium saccharin is carcinogenic for the urinary bladder of male and female rats, with male rats being more susceptible than female rats.

Saccharin given to rats induces hyperplasia, as well as carcinomas, of the urinary bladder. The development of these lesions was related to the time, as well as to the dose. Hyperplasia was present at 4 months as minimal foci, and increased in severity from slight to moderate and marked, as observed at 6, 12, and 18 months. Later polypoid hyperplasia, papillomas, early transitional cell carcinomas, and large carcinomas developed.

Hyperplasia and tumors of the urinary bladder were related to the dose at three different dose levels, and the lesions were present in higher numbers in male rats than in female rats. If rats with hyperplasia of the urinary bladder at 2 years had survived for a longer period of time, the incidence of tumors of the urinary bladder would have increased.

Bladder stones were never associated with tumors of the bladder. Some rats ingesting saccharin developed bladder stones, but not a single rat with a bladder stone had either hyperplasia or tumors of the urinary bladder. Parasites of the urinary bladder were not observed.

Lesions of the urinary bladder in rats ingesting saccharin cannot be related to the presence of urinary bladder stones or parasites as has been claimed in earlier studies using small numbers of rats. They are not caused by sodium.

**Weinberger Analysis of FDA Study (1973).** Dr. Morris A. Weinberger, Director, Division of Pathology, FDA, had the following comments and conclusions concerning the histopathology report of the FDA Study by Willigan (20, 22).

"Under these test conditions sodium saccharin induced transitional cell neoplasms of the urinary bladder in Charles River rats. The incidence of the neoplasms of the 75,000 group in comparison to the controls was statistically significant ( $p < 0.025$  using chi-square analysis)."

"Most of the carcinomas were classified by Dr. Willigan as transitional cell carcinomas (papillary and non-papillary). I generally concur in his findings."

"The histopathology, site of origin (dome of the bladder), morphologic characteristics, extent of in-

vasiveness, etc. of these neoplasms are remarkably similar to those that I reviewed in the WARF chronic toxicity study. They also resemble the cyclamate-induced urinary bladder tumors of the FDA study."

"Predisposing factors, e.g., bladder parasites, bladder stones, seminal vesicle casts, crystals, or crystal deposits were not seen to play a role in the genesis of the bladder neoplasms."

"The degree of malignancy of the tumors may be assessed from (i) morphologic characteristics, (ii) biologic behaviors. Morphologically, most of the tumors have been classified as transitional cell carcinomas."

**Canadian Health Protection Branch Study (1977).** Male and female Charles River Sprague-Dawley rats of the  $F_0$  generation, 50 per group and 30 days of age, ingested 2500 mg/kg-day sodium saccharin in a standard laboratory diet for 142 weeks (23, 24). Saccharin was produced by the Maumee-method and contained less than 0.2 ppm toluenesulfonamide. Male and female rats were mated after ingesting saccharin for approximately 90 days. Similar groups of rats which were exposed to saccharin *in utero* and during lactation as a result of maternal ingestion of saccharin ( $F_1$  generation) and later in the diet received saccharin for 127 weeks. Other rats that did not ingest saccharin served as controls.

Eight of 45 (18%)  $F_1$  male rats had malignant tumors and 4 of 45 (9%) had benign tumors of the urinary bladder (Table 15). Three of 38 (8%)  $F_0$  male rats developed malignant tumors and 4 of 38 (11%) developed benign tumors of the urinary bladder. There was a metastasis to a lymph node in at least one rat. Two rats with tumors of the urinary bladder had stones and four rats without tumors had stones. Parasites were not present in the urinary bladder. Small numbers of  $F_1$  female rats developed tumors of the urinary bladder (Table 16).

Male rats of the  $F_1$  generation developed more tumors of the urinary bladder than did those of the  $F_0$  generation. The tumors in the  $F_1$  male rats were more malignant and were observed earlier, 67 weeks compared to 87 weeks for  $F_0$  rats.

The increase in tumors of the urinary bladder are highly significant in male rats of both generations and in male and female rats of both generations (Table 17).

Of 172 rats ingesting saccharin, 21 rats developed tumors of the urinary bladder, compared to 1 of 163 controls ( $p = 0.000,00526$ ).

In summary, male rats ingesting saccharin in the diet developed tumors of the urinary bladder. Rats of the  $F_1$  generation were more susceptible than rats of the  $F_0$  generation.

Table 15. Tumors of the urinary bladder in male rats ingesting saccharin.

Dose, mg/kg-day	No. of tumors					
	F <sub>0</sub>			F <sub>1</sub>		
	Benign	Malignant	Total	Benign	Malignant	Total
0	1/36(3%)	0/36(0%)	1/36(3%)	0/42(0%)	0/42(0%)	0/42(0%)
2500	4/38(11%)	3/38(8%)	7/38(19%)	4/45(9%)	8/45(18%)	12/45(27%)
			( <i>p</i> = 0.0334)	( <i>p</i> = 0.0670)	( <i>p</i> = 0.003689)	( <i>p</i> = 0.000162)

Table 16. Tumors of the urinary bladder in female rats ingesting saccharin.

Dose, mg/kg-day	No. of tumors					
	F <sub>0</sub>			F <sub>1</sub>		
	Benign	Malignant	Total	Benign	Malignant	Total
0	0/38(0%)	0/38(0%)	0/38(0%)	0/47(0%)	0/47(0%)	0/47(0%)
2500	0/49(0%)	0/49(0%)	0/49(0%)	0/49(0%)	2/49(4%)	2/49(4%)

Table 17. Tumors of the urinary bladder in both sexes ingesting saccharin.

Dose, mg/kg-day	No. of lesions					
	F <sub>0</sub>			F <sub>1</sub>		
	Benign	Malignant	Total	Benign	Malignant	Total
0	1/74(1.35%)	0/74(0.00%)	1/74(1.35%)	0/89(0.00%)	0/89(0.00%)	0/89(0.00%)
2500	4/87(4.00%)	3/78(3.85%)	7/78(8.97%)	4/94(4.26%)	10/94(10.64%)	14/94(14.89%)
			( <i>p</i> = 0.0334)	( <i>p</i> = 0.0675)	( <i>p</i> = 0.001001)	( <i>p</i> = 0.000054)

**Chapman Study.** Chapman attempted to study the effect of parasites upon the induction of urinary bladder tumors by 2-acetylaminofluorene (25). The three strains of male and female rats used were Griedl (Steinhilber), Long-Evans, and Fischer 344. Some rats had urine dropped on them in order to infect them and others ingested 0.2% nitrofurantoin for 8 weeks in an effort to disinfect them.

Acetylaminofluorene, in a dose of 0.5% in ground Purina laboratory chow, was given to the rats at 4 months of age for 1 year in order to induce tumors of the urinary bladder. Some "paired groups" of two strains were given additional DL-tryptophan (1%) in a further attempt to induce tumors of the urinary bladder. An attempt was made to give some rats indole in the diet, but this was discontinued.

Initial numbers of rats in each group were not given, however, the results were based on the number of animals living over 7 months, i.e., 153 rats. The 20 different groups had from 0 to 15 rats in each group, with an average of 7.7 rats per group. There were no control rats not receiving acetylaminofluorene.

There were 5 bladder tumors: 3 of 77 males (4%) and 2 of 79 females (3%), or 5 of a total number of 156 rats (3%). One of the rats with a bladder tumor also ingested tryptophan. The incidence of tumors of the urinary bladder was assumed to be 0% in

animals not treated with chemicals. The tumors of the urinary bladder occurred in 5 of the 75 rats that had parasites. Bladder stones were not observed in rats of any group.

The results suggested that nitrofurantoin may have decreased the incidence of liver tumors and increased the numbers of rats with tumors of the lung or mammary gland. Nitrofurantoin may also have decreased the incidence of tumors of the urinary bladder. Tryptophan may have decreased the incidence of tumors of the mammary gland and lung.

The results were further complicated by problems associated with trying to infect and disinfect rats: "Our results in clearing infected rats of the worm were not completely successful. Of 52 infected rats fed nitrofurantoin in an attempt to clear them of infection, five were still infected when examined; only 37 of the 55 infected and untreated animals were infected at autopsy."

"The results from attempting to infest the clean animals were even less satisfactory. One of the 30 clean animals became infected spontaneously and only 3 of the 25 that were exposed to infection by feeding *T. crassicauda* eggs remained infected at the end of the experiment."

The authors also speculated that the low incidence of tumors of the urinary bladder, particularly

in rats given tryptophan, may have been related to the age of the rats at the start of the experiment, about 4 months.

In summary, the incidence of acetylaminofluorene-induced tumors of the urinary bladder was not increased significantly in rats with parasites of the urinary bladder. Additional groups of control rats receiving nitrofurantoin or tryptophan and not given acetylaminofluorene are necessary to properly interpret the data.

**Canadian Health Protection Branch Study (1977).** Male and female F<sub>0</sub> Charles River Sprague-Dawley rats, 30 days of age, 50 per group, ingested 0, 2.5, 25, or 250 mg/kg-day of *o*-toluenesulfonamide in the diet for 142 weeks (23, 24). The diet was adjusted weekly to maintain a constant level of *o*-toluenesulfonamide. Similar groups of rats, which were exposed to *o*-toluenesulfonamide *in utero* as a result of maternal ingestion, also received the chemical in the diet for 127 weeks. The levels of *o*-toluenesulfonamide were so chosen as to be equivalent to levels that rats may have consumed from the ingestion of saccharin-containing diets in the WARF and FDA Studies (19, 20).

Tumors of the urinary bladder were not increased significantly in rats ingesting *o*-toluenesulfonamide in the diet when compared to the controls (Tables 18, 19).

In this study, *o*-toluenesulfonamide did not induce tumors of the urinary bladder in rats.

## Mouse Studies

**Study of Allen et al.** A study was carried out in which pellets of saccharin and cholesterol were surgically implanted in the urinary bladder of unspecified sex and stock mice (26). Pellets weighing between 9 and 11 mg were prepared from a mixture of four parts cholesterol and one part saccharin. Control mice received pellets of cholesterol only.

Pellets containing saccharin induced a significant number of urinary bladder tumors (Table 20). Four of 13 mice (31%) had tumors of the urinary bladder, and two were carcinomas and one a papilloma ( $p = 0.04101$ ). One of 24 control mice (4%) had a carcinoma of the bladder.

In addition, the following points concerning the implantation of pellets into the urinary bladder of rodents were made.

The technique of implanting pellets into the urinary bladder of mice can be used to indicate whether the carcinogenic activity is caused by the chemical itself or by a metabolite, because the possibilities of metabolic changes occurring in the bladder are limited.

The urinary bladder of mice seem to be more re-

sistant to the apparent carcinogenic action of inert solids than are those of rats, so that mice may be more suitable animals for use with the implantation technique.

Although implantation of pellets of cholesterol only induces some tumors, the carcinogenicity of a particular chemical can be evaluated by the increase in the tumor incidence after implantation of a mixture of chemical and cholesterol.

The induction of tumors of the urinary bladder is related to the size of the pellet; therefore, the incidence of tumors in controls can be decreased by the use of smaller pellets.

In this study, saccharin induced tumors of the urinary bladder in mice. Most of the tumors were malignant.

**Study of Bryan et al.** Bryan et al. studied the carcinogenicity of sodium saccharin in mice by implanting pellets of saccharin and cholesterol in the urinary bladder (27). Pellets composed of sodium saccharin and cholesterol (1:4 mixture), weighing

Table 18. Tumors of the urinary bladder in male rats ingesting *o*-toluenesulfonamide.<sup>a</sup>

Dose, mg/kg-day	No. of tumors					
	F <sub>0</sub>			F <sub>1a</sub>		
	Benign	Malignant	Total	Benign	Malignant	Total
0	1/50	0/50	1/50	0/50	0/50	0/50
2.5	1/50	0/50	1/50	0/50	1/50	1/50
25	0/50	0/50	0/50	1/50	0/50	1/50
250	1/50	0/50	1/50	0/50	0/50	0/50

<sup>a</sup> Figures not corrected for survival time.

Table 19. Tumors of the urinary bladder in female rats ingesting *o*-toluenesulfonamide.<sup>a</sup>

Dose, mg/kg-day	No. of tumors					
	F <sub>0</sub>			F <sub>1a</sub>		
	Benign	Malignant	Total	Benign	Malignant	Total
0	0/50	0/50	0/50	0/50	0/50	0/50
2.5	1/50	0/50	1/50	2/50	0/50	2/50
25	0/50	0/50	0/50	0/50	0/50	0/50
250	0/50	0/50	0/50	0/50	0/50	0/50

<sup>a</sup> Figures not corrected for survival time.

Table 20. Number of mice treated with saccharin with tumors of the urinary bladder.

Treatment	No. of mice with tumors		
	Benign	Malignant	Combined
Cholesterol	0/24(0%)	1/24(4%)	1/24(4%)
Saccharin + cholesterol	1/13(8%)	3/13(23%)	4/13(31%) ( $p = 0.04101$ )

20–24 mg, were surgically placed in the urinary bladder lumens of two groups of 100 female Swiss mice 60–90 days old. Pure cholesterol pellets were placed in the urinary bladder of two groups of 100 mice each. This study was conducted at the same time as that reported previously with sodium cyclamate (29); therefore, the control groups given cholesterol alone were the same for both studies. To estimate the probable exposure of the urinary bladder to saccharin, pellets were removed from some mice at various times after implantation and the quantity of saccharin remaining in the pellets was measured (24). Surviving mice were killed after 13 months. All organs except the brain were inspected grossly, and histologic examination of representative tumors was done.

Of the saccharin, 50% had disappeared after 5.5 hr and 99% by 1.5 days. The first bladder carcinoma was observed in a mouse treated with saccharin that died after 293 days. Most mice survived for 326 days or longer. Mice exposed to sodium saccharin had significantly higher incidences of carcinomas of the urinary bladder (47% and 52%) than did the controls (13% and 12%) (Table 21). No other tissues of mice exposed to sodium saccharin were reported to have a tumor incidence significantly different from the controls; however, those data were not given.

Histologically, all of the transitional cell carcinomas infiltrated into the subepithelial connective tissue, and some infiltrated into the muscular wall, penetrated through the serosal layer of the bladder, or had local metastases in the pelvis.

In addition, the following points were made.

The carcinomas in mice exposed to sodium saccharin were often seen grossly and sometimes filled the lumen, were frequently multiple within the same bladder, were often more invasive into muscle ( $p < 0.009$ ), and were often undifferentiated and highly anaplastic.

The degree of histologic malignancy observed in the carcinomas of the urinary bladder induced by sodium saccharin was more severe than that seen in carcinomas induced by sodium cyclamate.

The carcinomas of the urinary bladder induced in mice by sodium saccharin were comparable to those

induced in mice, rats, and dogs by feeding the potent urinary bladder carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide, as well as other carcinogens.

In conclusion, female mice with pellets of sodium saccharin and cholesterol implanted in the urinary bladder developed highly significant incidences of undifferentiated and invasive carcinomas of the urinary bladder.

**Bio-Research Consultants.** Bio-Research Consultants carried out two separate studies with the same experimental design on the chronic effects of sodium saccharin in mice (14). Albino male and female mice (derived from HaM/ICR mice), 25 in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. The saccharin (Merck or Monsanto) was stated not to contain impurities. There were two groups of males and females for each dose level of saccharin.

Tumors of the urinary bladder were found in two male mice and papillary hyperplasia in three male mice ingesting saccharin. There was a tumor of the urinary bladder in one control mouse.

The results of this study suggest that larger numbers of mice ingesting saccharin are needed in order to determine its carcinogenicity for the urinary bladder.

**Other Studies.** Kroes, et al. gave Swiss-SPF male and female mice, 50 of each sex per group, 0%, 0.2%, or 0.5% saccharin in the diet for 21 months (30). Carcinomas of the urinary bladder were observed in one male mouse ingesting 0.2% and one male mouse ingesting 0.5% saccharin. Calculi were not associated with the carcinomas.

Amyloidosis of the kidney was increased in females receiving 0.5% saccharin.

The results of this study suggest that saccharin is carcinogenic for the urinary bladder of male mice. Saccharin should have been administered for a longer period of time. Amyloidosis and other diseases interfered with the health and lifespan and with the development of neoplasms.

## Summary

The findings are summarized in Table 22. In the earliest studies, small numbers of rats ingesting saccharin in the diet developed tumors of the urinary bladder. These studies did not demonstrate that saccharin was not carcinogenic for the urinary bladder, but strongly indicated that if studies were done using large numbers of rats saccharin most likely would be carcinogenic for the urinary bladder. There was no

Table 21. Number of mice treated with sodium saccharin with carcinoma of the urinary bladder.

Treatment	No. of mice with carcinomas		
	Group 1	Group 2	Groups combined
Cholesterol	8/63(13%)	5/43(12%)	13/106(12%)
Saccharin + cholesterol	31/66(47%)	33/64(52%)	64/130(49%)
	$(p = 0.000027) (p = 0.000029) (p = 0.000000003)$		

Table 22. Tumors of the urinary bladder in rats ingesting saccharin.

Study	Year	Dose, %	Animal	Number with tumors		p
				Control	Treated	
Lesell	1959	5.0	Rat (female)	0/20	2/20 (10%)	
Litton	1972	5.0	Rat (female)	0/20	1/26 (3.8%)	
FDA	1973	7.5	Rat (female)	0/24	4/31 (12.9%)	0.09226
			Rat (male)	1/25 (4%)	7/23 (30.4%)	0.01754
			Rat (both sexes)	1/49 (2%)	11/59 (18.6%)	0.005319
Warf	1973	0.05	Rat (female)	0/17	1/17 (5.9%)	0.500000
		5.0	Rat (male)	0/16	7/16 (44%)	0.003399
			Rat (both sexes)	0/33	8/33 (24.2%)	0.002417
Munroe (Canada)	1973	0.2	Rat (female)	0/56	1/56 (1.8%)	
		0.2	Rat (male)	0/57	1/51 (2%)	
		2.0	Rat (male)	0/57	2/52 (3.8%)	
			Rat (both levels, both sexes)	0/113	4/159 (2.5%)	0.11493
Hicks	1975	8	Rat (both sexes)	0/600 (0%)	3/138 (2%)	
Canada	1977	5.0	Rat (male)	1/78 (1.3%)	19/83 (22.9%)	0.00001263
			Rat (female)	0/85	2/89 (2.2%)	0.260
			Rat (both sexes)	1/163 (0.6%)	21/172 (12.2%)	0.00000526

evidence in these early studies that the tumors of the urinary bladder were caused by stones or by parasites in the urinary bladder and not by saccharin. There are no data showing that stones and parasites influence the induction of tumors of the urinary bladder in rats ingesting chemicals.

Recent studies have shown that saccharin is definitely carcinogenic for the urinary bladder in rats. Male rats are more susceptible than female rats. Rats of the  $F_1$  generation develop tumors more readily than do those of the  $F_0$  generation. The carcinomas are preceded by the development of mild, moderate, and severe hyperplasia, polypoid hyperplasia or hyperplastic nodules. Rats ingesting saccharin with carcinomas of the urinary bladder did not have bladder stones and rats with bladder stones did not have hyperplastic or neoplastic lesions of the urinary bladder. Neoplasms of the urinary bladder sometimes developed prior to the development of bladder stones in rats ingesting saccharin that were pretreated with nitrosamine. Parasites were not present in the urinary bladder of rats ingesting saccharin that had tumors of the bladder.

Those studies, indicating that saccharin most likely was carcinogenic for the urinary bladder in rats, were supported by the development of carcinomas of the urinary bladder in mice with surgically implanted saccharin cholesterol pellets. The results were so convincing that the route of administration of saccharin should not have been considered as not appropriate. Carcinomas of the urinary bladder of nitrosamine-pretreated rats ingesting sac-

charin also added to the accumulating evidence that saccharin was carcinogenic.

Rats ingesting saccharin developed chronic renal disease and tumors or polyps of the renal pelvic mucosa, a finding observed in the first study. It is apparent that a relationship exists between the dose of saccharin, chronic renal disease, renal pelvic tumors, and tumors of the urinary bladder; however, renal lesions in more recent studies have not been adequately examined. It appears that chronic renal disease results from the administration of low doses of saccharin and that tumors of the renal pelvis and urinary bladder develop with the higher doses. At lower doses saccharin may not reach the urinary bladder and could not be expected to induce hyperplastic or neoplastic lesions.

Renal pelvic lesions observed in the first, as well as in later, rat studies should have alerted investigators to the probability that tumors would also develop in the similar epithelium of the urinary bladder. These lesions should be considered as preneoplastic and as having the potential to become carcinomas.

Recent studies in rats ingesting saccharin with tumors of the urinary bladder also took into consideration other criticisms. Control rats were given sodium carbamate (at the same level as that ingested by the rats ingesting 5% sodium saccharin) and the saccharin contained fewer or no impurities. *o*-Toluenesulfonamide, a contaminant of saccharin in the earlier studies, is not carcinogenic for the urinary bladder of rats.

# Tumors Involving the Reproductive System

## Rat Studies

**Wisconsin Alumni Research Foundation (WARF) Institute Study.** Male and female Sprague-Dawley (F<sub>1</sub>) weanling rats, 20 per group, ingested 0%, 0.05%, 0.5%, or 5% sodium saccharin in Purina laboratory chow for 100 weeks (19).

Female rats developed increased incidences of both benign and malignant tumors of the reproductive system (ovary and uterus), which were dose related (Table 23). Such tumors were present in 6 of 20 female rats (30%) ingesting 5% sodium saccharin, compared to 1 of 17 control female rats (6%) ( $p = 0.0716$ ).

Malignant tumors were also increased, and they were dose related. There were 6%, 13%, and 20% ( $p = 0.0734$ ) in the saccharin-treated rats at the three doses; whereas none were seen in control females. The malignant tumors were squamous cell carcinomas of the uterus and carcinomas of the ovary.

In summary, the incidences of malignant tumors of the reproductive system were increased in female rats ingesting saccharin. Malignant tumors were present in the uterus and ovary.

**FDA Study (1973).** The FDA study used weanling Charles River Sprague-Dawley male and female rats ingesting 0%, 0.01%, 0.1%, 1%, 5%, or 7.5% sodium saccharin in Purina laboratory chow for approximately 28 months (31).

Rats received a thorough gross examination. Complete necropsies with histological examination were done unless autolysis was too advanced. Only tumors of the mammary gland were studied histologically, i.e., routine sections of mammary glands were not done in all rats.

Mammary tumors were seen in rats of both sexes living for 18 months or longer, and a few were also observed before 18 months (Tables 24 and 25). There were often two or more mammary tumors in female

**Table 23. Tumors of the reproductive system (uterus and ovary) in female rats ingesting saccharin.**

Dose, %	No. rats		total
	With benign tumors	With malignant tumors	
0	1/17( 6%)	0/17( 0%)	1/17( 6%)
0.05	0/17( 0%)	1/17( 6%) <sup>a</sup>	1/17( 6%)
0.5	1/15( 7%)	2/15(13%) <sup>a</sup>	3/15(20%)
5	2/20(10%)	4/20(20%) <sup>b</sup>	6/20(30%)
		( $p = 0.0734$ )	( $p = 0.0716$ )

<sup>a</sup> Squamous cell carcinomas of the uterus.

<sup>b</sup> Carcinomas of the ovary.

**Table 24. Tumors of the mammary gland in male rats ingesting saccharin.**

Dose, %	No. rats with mammary gland tumors		
	One/rat	Two or more/rat	Total
0	5/29(17%)	1/29( 3%)	6/29 (20%)
0.01	8/25(32%)	6/25(24%)	14/25 (56%)
0.1	9/27(33%)	0/27( 0%)	9/27 (33%)
1.0	8/27(30%) <sup>a</sup>	0/27( 0%)	8/27 (30%)
5.0	7/25(28%)	0/25( 0%)	7/25 (28%)
7.5	7/24(29%)	0/24( 0%)	7/24 (29%)
			45/128(35%)
			( $p = 0.0988$ )

<sup>a</sup> One rat had one mammary gland tumor prior to 18 months.

**Table 25. Tumors of the mammary gland in female rats ingesting saccharin.**

Dose, %	No. rats with mammary gland tumors		
	One/rat	Two or more/rat	Total
0	5/26(19%) <sup>a</sup>	1/26( 4%)	6/26(23%)
0.01	8/30(27%)	6/30(20%)	14/30(47%)
0.1	9/34(26%)	4/34(12%)	13/34(38%)
1.0	8/30(27%)	4/30(13%) <sup>b</sup>	12/30(40%)
5.0	7/27(26%)	5/27(18%) <sup>b</sup>	12/27(44%)
7.5	7/32(22%)	2/32( 6%)	9/32(28%)
0.01-7.5			60/153(39%)
			( $p = 0.0874$ )

<sup>a</sup> One rat had one mammary gland tumor prior to 18 months.

<sup>b</sup> Two rats had two or more mammary gland tumors before 18 months.

rats ingesting saccharin, whereas untreated female rats usually had one mammary gland tumor. The incidence of mammary gland tumors was highest in rats ingesting 0.01% saccharin; 56% in saccharin-treated males ( $p = 0.0038$ ), 20% in untreated males, 47% in treated females, and 23% in untreated females.

Sixty of 153 saccharin-treated female rats ( $p = 0.0874$ ) and 45 of 128 male rats ingesting saccharin ( $p = 0.0998$ ) developed tumors of the mammary gland.

It should be noted the incidence of tumors of the mammary gland would most likely have been higher if mammary glands of all rats had been examined histologically. There may have been tiny tumors ("microscopic") that were not seen on gross examination.

Furthermore, a thorough analysis of the benign versus malignant tumors of the mammary gland is needed. Malignant tumors generally occur more often in chemically treated rats than in untreated rats.

In summary, the incidences of tumors of the mammary gland were increased in rats ingesting saccharin. This finding is significant because saccharin was carcinogenic for the mammary gland and

not only in female rats, but also in male rats.

**Munroe et al. Study.** Munroe et al. (15) observed a sarcoma of the uterus in a female rat and adenocarcinomas in two female rats given 2430 mg saccharin/kg-day.

## Mouse Studies

**National Institute of Hygienic Sciences (Tokyo).** Male and female dde strain inbred mice ingested sodium saccharin in the diet. Mice ingested 0%, 0.2%, 1%, or 5% for 21 months (32). Many details are missing in the brief report.

There were increased numbers of tumors in the reproduction system (ovary and mammary gland). Six of 9 tumors (67%) in female mice ingesting 0.2%, 7 of 12 tumors (58%) in mice ingesting 1%, and 10 of 13 tumors (77%) in mice ingesting 5% saccharin were in the reproductive system. No distinction was made between benign and malignant tumors.

The number of mice with tumors of the ovary can be analyzed; however, the number of mice with tumors of the reproductive system cannot be analyzed because one mouse may have had more than one tumor.

Female mice ingesting saccharin had an increase incidence of tumors of the ovary, and the incidence was highest at the two largest doses (Table 26). Sixteen of 41 mice (40%) ingesting saccharin developed tumors of the ovary ( $p = 0.00347$ ).

In summary, the number of tumors in the reproductive system was increased in saccharin-treated female mice. The number of mice with ovarian tumors was also increased significantly in treated mice.

**Bio-Research Consultants Study.** Bio-Research Consultants carried out two separate studies with the same experimental design on the chronic effects of sodium saccharin in mice (14). Albino male and female mice, 25 in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. There were two groups of males and females for each dose level of saccharin.

Table 26. Tumors of the ovary in female mice ingesting saccharin.

Dose, %	No. of mice with tumors
0	0/14(0%)
0.2	3/18(17%)
1.0	7/11(64%)
5.0	6/12(50%)
All doses	16/41(40%)
	( $p = 0.00347$ )

The data from this study were analyzed at the NCI; it was concluded that "for tumors of the uterus among female mice the life table analysis reveals a significant effect in the high dose group for females" (13).

## Summary

Saccharin is carcinogenic for the reproductive system in female rats and mice and mammary gland in male rats.

Female rats ingesting saccharin developed increased incidences of tumors of the reproductive system, which were dose related. Both male and female rats ingesting saccharin developed increases in tumors of the mammary gland, as well as more tumors per rat. Saccharin-treated female mice had an increase in ovarian and uterine tumors.

## Tumors Involving the Hematopoietic System

### Rat Studies

**FDA Study (1948-1949).** Osborne-Mendel male and female weanling rats, 10 of each sex per group, ingested 0%, 0.01%, 0.1%, 0.5%, 1%, or 5% saccharin in the diet (33). Saccharin was mixed with ground commercial rat biscuits with 1% added cod-liver oil. Rats were kept in individual cages and given free access to the diets. Rats were killed after 2 yr. Detailed histological examinations were done for some rats, but others were limited to liver, kidney, and testis. Urinary bladder was not routinely examined.

"Lymphosarcoma" was present in the lung of 7 of 20 rats ingesting 5% saccharin. Both thoracic and abdominal lymphosarcomas were observed in 4 of the 7 rats. Three occurred in animals after 102 weeks. Lesions in rats were not attributed to the lower doses of saccharin. No mention is made of tumors in control rats.

Saccharin induced lymphosarcomas in rats. Other results are not complete because of limited histological examinations, failure to separate results by sex, and lack of correction for survival time.

**Long and Haberman (FDA Study 1948-1949).** Long and Haberman, with the assistance of Stewart, reviewed the available data and microscopic studies from the long-term rat feeding study on saccharin done at the FDA (8). Groups of 3-week-old Osborne-Mendel rats, 10 of each sex per group, received 0%, 0.01%, 0.1%, 0.5%, 1%, or 5% saccharin in the diet for 2 yr.

Lymphosarcomas were observed in increased in-

**Table 27. Numbers of male and female rats with lymphosarcoma.**

Dose, %	No. of lymphosarcomas		
	Thorax	Abdomen <sup>a</sup>	Total
0	0/20(0%)	0/20(0%)	0/20(0%)
0.01	8/14(57%)	0/14(0%)	8/14(57%)
0.1	5/16(31%)	0/16(0%)	5/16(31%)
0.5	2/15(13%)	0/15(0%)	2/15(13%)
1	1/18(6%)	0/18(0%)	1/18(6%)
5	7/17(41%) <sup>b</sup>	4/17(24%) <sup>b</sup>	7/17(41%)
Total			25/80(31.25%) ( <i>p</i> = 0.000053)

<sup>a</sup> Rats with lymphosarcomas of the abdomen also had lymphosarcoma of the thorax.

<sup>b</sup> One rat also had lymphocytic leukemia.

cidences in the thoracic and abdominal cavity of saccharin-treated rats when compared to the controls (*p* = 0.000053) (Table 27). It should be noted that untreated and treated rats of all groups had only a slight degree of acute and chronic pneumonia.

The increased incidences of lymphosarcoma of the thorax in rats at the 0.01% and 5%, as well as for both abdominal and thoracic lymphosarcomas in rats at the 5% dose of saccharin, are highly significant when compared to the control rats in the saccharin study. The incidences are significant also when compared with the pooled controls, those used for the saccharin study, and the controls for three other artificial sweeteners. Abdominal lymphosarcomas were found only in rats treated at the 5% dose level and not in matched or pooled controls. There is a progressive dose-related increase in the incidence of thoracic lymphosarcomas from the 1% to the 0.01% treated rats, i.e., from 6% to 57%.

Not only was there an increased incidence of lymphosarcomas in the rats receiving 5% saccharin, but there was also an increased incidence of hyperplasia (increased cellularity) of the bone marrow in those rats (91% versus 66% to 83% for the various control groups).

It was reported that there were no sex differences in the incidences of thoracic tumors; however, in some groups on control rats used at FDA for testing of chemicals, the incidence was as low as 0% in untreated male rats and somewhat higher in untreated female rats. If that is the case for the controls in this study, the incidences in the saccharin-treated male rats could even be higher than those given here for males and females combined.

In conclusion, saccharin is a carcinogen for lymphocytic tissue in the thoracic cavity, as well as in the abdominal cavity. Rats also had hyperplasia of the bone marrow.

**Study of Schmähl.** Schmähl gave 70- to 90-day-old male and female rats 0%, 0.2%, or 0.5% sodium saccharin in the diet for a lifetime (11). Rats

also received the much higher doses of 7% or 5% of cyclamate. This strain of rats was described as being susceptible to the development of tumors of the urinary bladder.

Histologic examination of tissues, with the exception of the bladder, were done "when organs seemed pathologically changed." Only grossly observable tumors, and not microscopic tumors, were examined. Every rat in the study was considered at risk, i.e., survived long enough to develop a tumor. The tumor incidences for the sexes were combined rather than being analyzed separately.

The analysis of malignant tumors in the various groups of rats is complicated by the 12% incidence of malignant tumors in the control rats. Lymphomas and leukemias were found in 14% of treated rats and 4% of control rats.

It is well known that animals that die early from infectious diseases or that are not healthy develop fewer, if any, tumors. The statement was made that "most of the animals died from typical infections of the respiratory tract." Indeed, another study of carcinogenicity of a chemical from the same Institute went even further to say that the low incidence of tumors might be related to the high incidence of infections (34).

In summary, rats ingesting sodium saccharin, particularly at the 0.5% level, developed slightly increased incidences of lymphomas and leukemia.

The results obtained were under the conditions of this experiment (incomplete histology, failure to separate the results by sex, including all rats at risk for the development of tumors, and using a strain with a high incidence of "spontaneous" tumors). Furthermore, "most of the animals died from typical infections of the respiratory tract."

**Study of Munroe et al.** Munroe et al. studied the carcinogenicity of commercial saccharin in male and female Charles River weanling rats weighing 50-60 grams (15). Groups of 60 male and 60 female rats were given sodium saccharin, 0, 90, 270, 810, or 2430 mg/kg-day in a synthetic diet for 26 months. The urinary bladder, as well as "other grossly abnormal organs," were examined histologically.

Particular attention was given to regional pelvic and lower abdominal lymph nodes because of the possibility of metastases from urinary bladder tumors; however, only other grossly enlarged lymph nodes or enlarged spleens were examined histologically.

There were increased incidences of lymphoma or leukemia in the saccharin-treated male rats (Table 28). Of control male rats, 3.5% developed lymphoma or leukemia, whereas 9% of the 270 mg/kg dose level male rats, and 13% of the 2430 mg/kg dose level developed lymphoma or leukemia. Large



**Table 28. Male rats ingesting saccharin with lymphoma or leukemia.<sup>a</sup>**

Dose, mg/kg	No. rats with lymphoma or leukemia
0	2/57(3.5%)
90	2/51(4%)
270	5/54(9%)
810	2/5 (4%)
2430	7/54(13%)
	( $p = 0.06873$ )

<sup>a</sup> The effective number of rats, or rats that survived long enough to develop tumors, is not given. Only about 40 rats per group were alive at 18 months.

numbers of rats of all groups were also diagnosed as having "chronic lymphadenitis/reactive hyperplasia."

In this study, male rats ingesting saccharin developed slightly increased incidences of lymphoma or leukemia.

## Summary

Saccharin is carcinogenic for lymphocytic tissue in rats. Lymphosarcomas were observed in significantly increased incidences in the thoracic and abdominal cavity of saccharin-treated rats. Rats ingesting saccharin developed slightly more lymphomas and leukemias than did untreated rats.

## Tumors Involving the Lungs and the Vascular System

### Rat Studies

**FDA Study (1948-1949) and Long and Haberman.** Lymphosarcomas were observed in the lung and abdominal cavity of saccharin-treated rats (8, 33). Lymphosarcomas of the lung were increased in rats ingesting 0.01% or 5% saccharin, when compared to the control rats, as well as in all treated rats.

These tumors have been discussed with tumors of the hematopoietic system.

### Mouse Studies

**Bio-Research Consultants.** Bio-Research Consultants carried out two separate studies with the same experimental design on the chronic effects of sodium saccharin in mice (14). Albino male and female mice (derived from HaM/ICR mice), 25 of each sex in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. The saccharin (Merck or Monsanto) was

stated to contain no impurities. There were two groups of males and females for each dose level of saccharin.

Animals dying during the first 6 months were not autopsied. Thereafter, complete gross autopsies were done unless mice were too autolyzed for histological examination. Histopathological examination was done on "grossly pathological organs of all animals and on all vital organs in at least 12 animals in each group."

There were increased incidences of tumors of the lung in the male mice ingesting 1% sodium saccharin ( $p = 0.006566$ ). Benign and malignant tumors of the lung should also be evaluated, because malignant tumors are more likely to be seen in treated mice and benign tumors in controls. Four of 14 male mice (29%) in one group had carcinomas of the lung, compared to 1 of 19 controls (5%) (Table 29).

There was an increase in the incidence of vascular tumors in some groups of male mice ingesting sodium saccharin. The mice ingesting 5% had 32% hemangiomas and 11% hemangiosarcomas ( $p = 0.0327$ ); those ingesting 1% had 7% hemangiosarcomas, compared to 5% hemangiomas and 0% hemangiosarcomas in the control male mice (Table 30).

In summary, male mice ingesting saccharin had increased incidences of vascular tumors, and of tumors of the lung. Vascular tumors in treated mice were sometimes malignant.

## Summary

Saccharin is carcinogenic for the lung and vascular system in mice. Male mice ingesting saccharin had increased incidences of vascular tumors and of tumors of the lung. Vascular tumors in treated mice

**Table 29. Tumors of the lung in male mice ingesting sodium saccharin in both studies.**

Dose, %	No. of mice		
	Group 1	Group 2	Total
0			2/19(11%)
1	6/14(43%)	8/15(53%)	14/29(48%)
			( $p = .006566$ )
5	4/15(27%)	5/19(26%)	9/34(27%)

**Table 30. Tumors of the vascular system in male mice ingesting sodium saccharin in both studies.**

Dose, %	No. of mice		
	Group 1	Group 2	Total
0			1/19(5%)
1	1/14(7%)	1/15(7%)	2/29(7%)
5	2/15(13%)	8/19(42%)	10/34(29%)
			( $p = 0.0327$ )

were sometimes malignant. Rats ingesting saccharin developed lymphosarcomas of the lung.

## Tumors Involving Squamous Epithelium

### Rat Studies

Bio-Research Consultants carried out two separate studies with the same experimental design on the chronic effects of sodium saccharin in rats (14). CDI random-bred male rats, 25 of each sex in each group, ingested 9%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. The saccharin (Merck or Monsanto) was stated not to contain impurities. There were two tumors of the forestomach and one of the skin in rats on the high dose of sodium saccharin (12%) and two tumors of the stomach in rats on the low dose (8%), whereas control rats did not develop such tumors. There were tumors in the stomach and skin of saccharin-treated rats; none were seen in controls. There also were tumors in the stomach and skin of mice given saccharin at the same time.

Tumors of the skin were also observed in rats in the National Institute of Hygienic Sciences study (39).

### Mouse Studies

**Salaman and Roe Tumor-Initiating Study in Mice.** There are chemicals which, though not carcinogenic for mouse skin, produce changes in the skin that develop into tumors after a subsequent short course of croton oil applications (35). Salaman and Roe studied the initiating action of saccharin on the induction of skin tumors by croton oil. "S" strain mice, 20 per group, were used.

Saccharin was administered (8% solution in acetone or methanol) by skin painting, as was croton oil. Ten "thrice weekly" dermal applications of saccharin were given for a total dose of 0.24 g. There was a 25-day interval after the first application of test substance and the first application of croton oil. Weekly applications of 0.25% croton in acetone were given for 18 weeks.

At the end of the treatment with croton oil, 7 of 20 mice (35%) had skin tumors and a total of 14 tumors was found. There were a few papillomas of the skin in mice in two of three control groups (1 of 17, 4 of 19, and 0 of 17, or 5 of 53). The authors pointed out: "Although treatment with croton oil without an initiator gives some skin tumors in mice, the carcinogenicity of an initiator can be evaluated by the increase in the tumor incidence after treatment with an initiator."

In summary, saccharin caused skin tumors to develop in mice when its application was followed by treatment with croton oil.

**Bio-Research Consultants.** Bio-Research Consultants carried out two identical studies with the same experimental design on the chronic effects of sodium saccharin in mice (14). Random-bred albino male and female mice, 25 in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. The saccharin (Merck and Monsanto) was stated as containing no impurities. There were two groups of males and females for each dose level of saccharin.

Male mice developed some tumors of the stomach or skin. Four of 29 mice (14%) ingesting 1% sodium saccharin had such tumors, compared to 0% of the control mice. One tumor was a squamous cell carcinoma of the forestomach and another an adenocarcinoma of the stomach in mice ingesting the low dose of saccharin. Occasional mice in both treated and control groups had atypical hyperplasia of the glandular mucosa of the stomach, but numbers were not given and its significance cannot be evaluated.

In this study, tumors of the stomach and skin were present in some mice ingesting saccharin, a finding similar to that in rats given saccharin at the same time.

**Roe et al. Tumor Promoting Study.** This study was concerned with the effect of sweeteners on benzopyrene-induced tumors of the forestomach in female albino mice (36). Mice, 50 per group, received gastric instillation of 50  $\mu$ g of BP in 0.2 ml PEG after overnight starvation. After 7 days, mice ingested 5% saccharin in the diet until they were killed 18 months after receiving benzopyrene. Controls received PEG only, followed by saccharin.

Because of a misunderstanding, mice were not allocated randomly to the eight treatment groups. The oldest, and therefore heaviest mice, were included mainly in the control groups (groups 1 and 2) while the lightest and youngest mice were allocated to test groups 3-8.

Necropsis were carried out on mice killed or found dead. All tumors or lesions suspected of being tumors, were studied histologically.

In this study, saccharin did not have a tumor-promoting effect on tumors of the forestomach induced by benzopyrene.

### Summary

Rats and mice ingesting saccharin tended to have tumors of the skin or stomach; whereas none were seen in untreated controls. Saccharin was a tumor initiator, i.e., skin tumors developed in mice when

its application was followed by treatment with croton oil.

## Tumors Involving All Organs

### Rat Studies

**Wisconsin Alumni Research Foundation (WARF) Institute Study.** The WARF Institute (International Sugar Research Foundation) described the results of a study on Sprague-Dawley (F<sub>1</sub>) weanling rats weighing 45-55 g (19). F<sub>1</sub> were the litters of female rats given saccharin. Treated rats were exposed to saccharin from inception. Male and female rats, 20 per group, ingested 0%, 0.05%, 0.5%, or 5% sodium saccharin in Purina laboratory chow *ad libitum* for 100 weeks. Sodium saccharin was obtained from Mallinkrodt Chemical Works. Purina laboratory chow contained 23% protein, 4.5% fat, 6.0% crude fiber, and 9.0% ash. Rats were housed in metal screen bottom cages. Most tissues were sectioned histologically, with the exception of mammary gland and pituitary, unless they had grossly observable tumors.

The report concluded that there were increased incidences of benign and malignant tumors, as well as malignant tumors, in male rats ingesting 5% sodium saccharin. Fourteen of 16 rats (87%) ingesting saccharin developed tumors, compared with 3 of 16 control rats (19%) ( $p = 0.000122$ ). Malignant tumors were found in 11 of 16 treated rats (69%) and 1 of 16 control rats (6%) ( $p = 0.000318$ ) (Table 31).

The incidence of malignant tumors was also slightly increased in female rats ingesting 5% sodium saccharin (45%); however, the incidence of malignant tumors in control rats was 23%.

The conclusions stated that "of the tumors found, the adenomas and adenocarcinomas of the pituitary, adenomas of the thyroid, squamous cell carcinomas of the uterus, and transitional cell carcinomas of the urinary bladders were higher in the test than in the controls . . ." Squamous cell carcinomas of the uterus, carcinomas of the ovary, and transitional cell carcinomas of the urinary bladder were found only in the treated rats and not in the untreated rats. There was a dose-related response for carcinomas of the reproductive system.

Table 31. Incidence of benign and malignant tumors in male rats ingesting saccharin.

Dose, %	No. of rats		
	Benign tumors	Malignant tumors	Total with tumors
0	2/16(13%)	1/16(6%)	3/16(19%)
5	3/16(19%)	11/16(69%)	14/16(87%)
		( $p = 0.000318$ )	( $p = 0.000122$ )

In conclusion, sodium saccharin is carcinogenic in male rats. There were significant increases of benign and malignant tumors and of malignant tumors only. There also were carcinomas of the reproductive system, for which there was a dose-related response in treated female rats.

**FDA Study (1973).** Male and female Charles River Sprague-Dawley rats, F<sub>1</sub> generation, 48 per group, ingested 0%, 0.01%, 0.1%, 1%, 5%, or 7.5% sodium saccharin (Monsanto) in the diet (20). Some rats were killed after 14 and 18 months, and the remaining rats after 28 months. Control rats were given a diet containing sodium carbonate at the same level as that ingested by the rats ingesting 5% sodium saccharin. Rats given 0.01% saccharin gained more weight than control rats or rats receiving the other doses of saccharin.

There were increased incidences of malignant tumors at all sites in rats ingesting the higher doses of saccharin for 24 months or more (Table 32). The tumors were related to the dose of saccharin. Malignant tumors were found in 8 of 26 male rats (36%) ingesting 7.5% saccharin compared to 2 of 29 control male rats (7%) ( $p = 0.025$ ); 7 of 29 female rats (24%) given 5% ( $p = 0.0327$ ); and in 9 of 32 female rats (28%) given the 7.5% ( $p = 0.0131$ ), compared to 1 of 27 female control rats (40%).

If all saccharin-treated rats are considered together, 16% of treated male rats compared with 7% of untreated males, and 17% of treated female rats and 4% of control females, had malignant tumors. Tumors were found in the urinary bladder, mammary gland, and in other organs as well.

In summary, saccharin caused an increase in malignant tumors at all sites in male and female rats ingesting saccharin, particularly at the highest doses.

**Bio-Research Consultants Study.** Bio-Research Consultants carried out two separate studies with the same experimental design on the chronic effects of sodium saccharin in rats (Charles River derived from Sprague-Dawley) (14). The only variable in the two studies was the source of saccharin. CDI random-bred male rats, 25 in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer. The saccharin (Merck or Monsanto) was stated not to contain impurities.

Animals dying during the first 6 months were not necropsied. Thereafter, complete gross necropsies were done unless rats were too autolyzed for histological examination. Histopathological examination was done on "grossly pathological organs of all animals and on all vital organs in at least 12 animals in each group." Neck organs were not included in the tissues examined.

There was an increased mortality after 1 year in

Table 32. Number of rats ingesting varying dose levels of saccharin with malignant tumors at all sites.

Dose, %	No. of rats with tumors		
	Male rats	Female rats	Both sexes with tumors
0	2/29(7%)	1/27(4%)	3/56(5.4%)
0.01	2/28(7%)	3/30(10%)	5/58(8.6%)
0.1	5/29(14%)	3/32(9%)	8/61(13.1%)
1	3/28(11%)	5/32(16%) $p = 0.141$	8/60(13.3%) $p = 0.124997$
5	4/24(17%) $p = 0.247$	7/29(24%) $p = 0.0327$	11/53(20.8%) $p = 0.016050$
7.5	8/26(36%) $p = 0.025$	9/32(28%) $p = 0.0131$	17/58(29.3%) $p = 0.000647$
All levels	22/135(16.3%) ( $p = 0.155760$ )	27/155(17.4%) ( $p = 0.051257$ )	49/290(16.90%) ( $p = 0.015698$ )

one group of saccharin-treated male rats. Occasional high calcium and phosphorus values were found in some rats.

If tumors in organs other than the pituitary, a commonly occurring tumor in control rats of this strain, are considered, 17 of 28 male rats (61%) ingesting the low dose of sodium saccharin had tumors; whereas 4 of 16 control male rats (25%) had tumors ( $p = 0.02454$ ) (Table 33). Seven of 28 male (29%) on the low dose of sodium saccharin had malignant tumors compared to 2 of 16 control rats (12%).

In this study, tumors in organs other than the pituitary, a tumor commonly seen in controls, were increased in rats on the low dose of saccharin. There were tumors in the stomach and skin of saccharin-treated rats; whereas none were seen in controls. There also were tumors in the stomach and skin of mice given saccharin at the same time.

**Litton Bionetics Study.** Litton Bionetics studied the chronic effects of sodium saccharin in Charles River CD male and female rats (12). Groups of 20 rats of each sex ingested 1% or 5% sodium saccharin in the diet for periods up to 2 years. Groups of 20 rats of each sex did not ingest saccharin. Complete necropsies were carried out. There were two duplicate studies, which were referred to as Saccharin I and Saccharin II.

The data from these studies was analyzed at the NCI and the following conclusions were drawn.

"The only significant differences noted were in the Saccharin II experiment. Here in the female rats, the saccharin-fed rats (both doses) had a significantly higher proportion of tumorous rats . . . with  $p = 0.02$ ." (13)

Table 33. Number of male rats ingesting saccharin with tumors other than pituitary in both studies.

Dose, %	No. of rats		
	Group 1	Group 2	Total
0			4/16(25%)
1	8/13(62%)	9/15(60%)	17/28(61%)
5	5/12(42%)	4/14(29%)	9/26(34%)
			( $p = 0.02454$ )

In summary, female rats ingesting saccharin developed an increase in tumors at all sites when compared with the controls.

**National Institute of Hygienic Sciences (Tokyo).** Sodium saccharin (Taiyo Chemistry Industry) was given to Sprague-Dawley male rats in the diet for 28 months (37). Groups of 54 rats ingested sodium saccharin on the following schedule: 2% day 0 to 20, 3% day 21 to 60, 4% day 61 to 150, and 5% after 150 days. Ten of 16 rats in each group were killed after 12 months. Many details are not included in a brief report.

Eight of 41 control rats were dead by 18 months, 17 of 41 by 24 months, and 27 of 30 by 28 months. Five of 42 saccharin-treated rats were dead by 18 months, 16 of 42 by 24 months, and 25 of 31 by 28 months.

Tumors were found mainly in the skin of both treated and untreated rats, as well as the lung in treated rats. Six of 42 rats (14%) ingesting saccharin and 3 of 41 control rats (7%) had skin tumors; 0% control rats and 5% of rats ingesting saccharin had lung tumors. No distinction was made between benign and malignant tumors.

In summary, saccharin-treated male rats had two times as many tumors as control rats. The tumors were located mainly in the skin and lung.

**Lessel Study.** Boots-Wistar rats, 20 per group, ingested 0%, 0.005%, 0.05%, 0.5%, or 5% saccharin in the diet for 2 years (5, 6).

There were increases in benign and malignant tumors in male rats ingesting 0.05% (46%) or 0.5% (31%) saccharin for 18 months or longer compared to 16% in the controls. The incidence of tumors in control female rats was high. Lymphomas were present in one control, one rat ingesting 0.005%, and two rats ingesting 0.5 saccharin. A few rats had lesions in the urinary bladder.

In this study, there were slight increases in the incidence of benign and malignant tumors in male rats ingesting saccharin.

**Study of Munroe et al.** Munroe et al. studied the carcinogenicity of commercial saccharin in male and female Charles River weanling rats weighing

50–60 g. Groups of 60 male and 60 female rats were given 0, 90, 270, 810, or 2430 mg/kg-day sodium saccharin in a synthetic diet for 26 months (15).

Some rats developed leukemia and lymphoma.

There were also unusual or rare tumors observed in treated rats but not in the control rats. These tumors were one hepatocellular carcinoma of the liver in a male given 270 mg/kg, two cholangiocarcinomas of the liver in females given 2430 mg/kg, an adenocarcinoma of the prostate in a male given 270 mg/kg, one malignant mixed mesenchymal tumor of the uterus in a female given 2430 mg/kg, and two endometrial adenocarcinomas of the uterus in females given 810 mg/kg and 2430 mg/kg.

**Canadian Health Protection Branch Study (1977).** Male and female Charles River Sprague-Dawley rats of the  $F_0$  generation, 50 per group and 30 days of age, ingested sodium saccharin, 2500 mg/kg-day in a standard laboratory diet for 142 weeks (23). Saccharin was produced by the Maumee-method and contained less than 0.2 ppm toluenesulfonamide. Male and female rats were mated after ingesting saccharin for approximately 90 days. Similar groups of rats which were exposed to saccharin *in utero* and during lactation as a result of maternal ingestion of saccharin ( $F_1$  generation) and later in the diet received saccharin for 127 weeks. Other rats that did not ingest saccharin served as controls.

Lymphoma/leukemia, pheochromocytoma of the adrenal, and biliary cystadenomas of the liver were slightly increased in saccharin-treated  $F_1$  generation female rats (Table 34). Two saccharin-treated female rats of the  $F_0$  generation had squamous cell carcinoma of the renal pelvis.

There was a squamous cell carcinoma of the renal pelvis in one saccharin-treated male rat of the  $F_0$  generation; a papilloma of the urethra in one male rat given saccharin and a squamous cell carcinoma of the urethra in one male rat given saccharin, both in the  $F_1$  generation.

**Table 34. Benign and malignant tumors in female rats of the  $F_1$  generation.**

Tumor	Control	Saccharin-treated
<b>Benign</b>		
Myxoma, heart	0	1
Adenomatous polyp, stomach	0	1
Biliary cystadenoma, liver	1/50 (2%)	5/50 (10%)
Cortical adenoma, adrenal	2	4
Pheochromocytoma, adrenal	3/50 (16%)	7/50 (14%)
Intraductal papilloma, kidney	0	1
Meningioma, meninges	0	2
<b>Malignant</b>		
Lymphoma/leukemia	7/50 (14%)	12/50 (24%)
Cortical adenocarcinoma, adrenal	0	1
Leiomyosarcoma, small intestine	0	1

There was extensive pelvic epithelial hyperplasia in the kidney of 11 of 49 control and 24 of 48 saccharin-treated  $F_0$  generation male rats and 10 of 49 control and 32 of 49 male rats given saccharin in the  $F_1$  generation. Extensive intraepithelial calcification of the kidney was seen in 3 control and 22 treated male rats of the  $F_0$  generation and 4 control and 19 treated male rats of the  $F_1$  generation. Seven of 50 control and 32 of 50 saccharin-treated  $F_1$  generation female rats had extensive pelvic epithelial hyperplasia.

## Mouse Studies

**Bio-Research Consultants.** Two comparable studies with the same experimental design were carried out. Saccharin was obtained from two sources (Merck or Monsanto). Random-bred albino male and female mice (derived from HaM/ICR mice), 25 in each group, ingested 0%, 1%, or 5% sodium saccharin in the diet for 104 weeks or longer (14). The mice were 8 weeks old at the start of the study.

Animals dying during the first 6 months were not autopsied. Thereafter, complete gross autopsies were done unless mice were too autolyzed for histological examination. Histopathological examination was done on "grossly pathological organs of all animals and on all vital organs in at least 12 animals in each group."

There was an increased incidence of tumors at all sites in the males ingesting 1% ( $p = 0.001581$ ) and 5% ( $p = 0.005228$ ) sodium saccharin in each study (Table 35). In addition, there were increased incidences of tumors of the lung in the male mice ingesting 1% sodium saccharin, and tumors of the vascular system in treated male mice.

In summary, there was an increase in tumors in all organs in male mice ingesting saccharin. Saccharin was also carcinogenic for the pulmonary and vascular systems in male mice.

**National Institute of Hygienic Sciences (Tokyo).** Male and female dde strain inbred mice, 50 in each group, ingested sodium saccharin (Taiyo Chemical Industry) in the diet for 21 months (32). The doses administered were 0%, 0.2%, 1%, or 5%. Many details are not included in the brief report.

**Table 35. Tumors in male mice ingesting saccharin in both studies.**

Dose, %	No. of mice		
	Group 1	Group 2	Total
0			4/19(21%)
1	10/14(71%)	10/15(67%)	20/29(69%)
			( $p = 0.001581$ )
5	9/15(60%)	12/19(63%)	21/34(62%)
			( $p = 0.005228$ )
			41/63(65.1%)
			( $p = 0.000912$ )

Five mice from each group were killed at the end of 12 or 18 months.

There were no data concerning the total number of mice with tumors. Only total numbers of tumors were given. Total numbers of tumors were increased in female mice ingesting all three doses of sodium saccharin for 21 months. There were 0 tumors in 14 female control mice, 9 tumors in 18 female mice ingesting 0.2%, 12 tumors in 11 mice ingesting 1%, and 13 tumors in 12 mice ingesting 5%.

The data are not clear as to the number of mice with tumors and total number of all tumors. An analysis of the incidence of mice with tumors in any organ cannot be carried out. There was, however, a significant increase in the number of mice ingesting saccharin with ovarian tumors.

**Study of Roe et al.** The study of Roe et al. was concerned with the effect of saccharin on benzopyrene-induced (BP) tumors of the forestomach in female albino mice (36). Control mice received a single gastric instillation of the BP vehicle, PEG, followed by the ingesting of 5% saccharin. Mice were killed 18 months after the start of the experiment.

"Because of a misunderstanding, mice were not allocated randomly to the eight treatment groups. The oldest, and therefore heaviest, mice were included mainly in the control groups (groups 1 and 2) while the lightest and youngest mice were allocated to test groups 3-8."

Postmortem examinations were carried out on mice killed or found dead. All major organs, including the urinary bladder, but excluding the cranium, were examined grossly. All tumors, or lesions suspected of being tumors were studied histologically. Urinary bladders were not sectioned histologically.

Tumors were not increased in mice ingesting saccharin; however, results must be reexamined because of the different ages for control and saccharin-treated mice. Subcutaneous sarcomas, sarcomas of the uterus, and undifferentiated lung tumors were observed in older control mice and not in younger mice.

**Study of Kroes et al.** Kroes et al. gave Swiss-SPF male and female mice, 50 of each sex per group, 0%, 0.2%, or 0.5% saccharin (Bayer Farma, N. V., Amsterdam) in the diet for 21 months (30). Large numbers of mice died before 18 months.

The results stated that the incidence of amyloidosis of the kidney was increased in females receiving 0.5% saccharin. Leukemia and "proliferative alterations in the lungs" were frequently observed. The data concerning controls and saccharin-treated mice was not given.

Swiss-SPF mice are not suitable for long-term carcinogenicity studies. Amyloidosis of the kidneys, as well as other diseases, interfere with their health and shorten their life span.

No conclusions concerning the carcinogenicity of saccharin, other than the urinary bladder, can be made from this study.

## Other Studies

**Study of Althoff et al.** Saccharin (Sigma Chemical Co., St. Louis, Missouri) was administered to male and female Syrian golden hamsters (38). Groups of 30 males and 30 females received 0%, 0.156%, 0.312%, 0.625%, or 1.25% saccharin in the drinking water. Average survival was from 50 to 60 weeks.

The results include only a list of the tumors found in the hamsters in the study. There were three tumors in treated females; whereas no tumors were seen in the control females. In addition, "generalized amyloidosis and vascular calcinosis, common in animals over 40 weeks of age, were found in both untreated and treated hamsters. The incidence in all treated animals was higher than in contemporary controls. . . ." No mention is made of chronic renal disease, although there were adenomas of the parathyroid glands.

No conclusions concerning the carcinogenicity of saccharin can be drawn from this subchronic study. These hamsters are not suitable for long-term carcinogenicity studies. Amyloidosis, vascular calcinosis, and other diseases interfere with their health and shorten their life span. Furthermore, the study was primarily concerned with low dose levels. Saccharin was administered in the water.

**Study of McChesney et al.** Rhesus monkeys, male and female were given soluble saccharin, 0, 20, 100, or 500 mg/kg-day 6 days a week for 79 months (39, 40). There were two or three monkeys in each group. There were two deaths in the control group and one in each of the experimental groups, which were not attributed to the administration of saccharin.

No conclusions concerning carcinogenicity of saccharin can be made from this study. The data is given in abstract form. Six and one-half years is not considered as sufficient for a carcinogenicity study in monkeys.

## Summary

The findings are summarized in Table 36.

Early studies suggested that tumors at all sites, particularly malignant tumors, might be increased in rats ingesting saccharin in the diet. Later studies

Table 36. Tumors involving all organ systems.

Study	Year	Dose, %	Type of tumor	Animal	Number (%) with tumors		
					Controls	Treated	<i>p</i>
Warf	1973	5	Benign	Rat (male)	2/16(13%)	3/16 (19%)	
			Malignant		1/16( 6%)	11/16 (69%)	(0.000318)
			Benign and malignant		3/16(19%)	14/16 (87%)	(0.000122)
FDA	1973	5	Malignant	Rat (male and female)	3/56(5.4%)	11/53 (21%)	(0.016050)
		7.5				17/58 (29.3%)	(0.000647)
		0.01-7.5				49/290(17%)	(0.015698)
Bio-Research Consultants	1973	1	Benign and malignant	Mouse (male)	4/19(21%)	20/29 (69%)	(0.001581)
		5				21/34 (62%)	(0.005228)
		1 + 5				41/63 (65%)	(0.000912)

disclosed that, indeed, benign and malignant tumors, or malignant tumors alone, were increased in male and female rats and in male mice ingesting saccharin. Male rats were more susceptible to the development of malignant tumors than were female rats, more so at the highest doses. Unusual malignant tumors were sometimes found in saccharin-treated rats and not in the controls.

There also were increased incidences of tumors at specific sites, i.e., specific organ systems. Tumors were seen chiefly in the urinary, reproductive, and hematopoietic systems, and also in the lungs, vascular system, and squamous epithelium.

## Comments

The arguments for ignoring the results of the tumors developing in the urinary bladder were: (1) small numbers of rats with tumors; (2) tumors were caused by stones in the urinary bladder; (3) tumors were caused by parasites in the urinary bladder; (4) tumors were not dose-related and occurred only at the highest doses; (5) tumors were caused by the impurity, *o*-toluenesulfonamide, and not by saccharin; (6) tumors developing in the bladder implantation study and certain other studies were irrelevant; (7) tumors of the urinary bladder were not preceded by the development of preneoplastic lesions and were not very malignant; (8) studies were not well planned; (9) tumors did not develop in organs other than the urinary bladder; and (10) studies have shown saccharin to be safe for humans.

### Small Numbers of Rats with Tumors

Studies with small numbers of rats ingesting saccharin with tumors of the urinary bladder were readily dismissed, even though some stocks of untreated rats did not develop such tumors. The results of such studies were interpreted as proving

that saccharin was not carcinogenic for rats. The results actually indicated that saccharin would probably be carcinogenic if large enough numbers of animals had been used in the studies.

Rats ingesting saccharin also developed significant numbers of tumors or polyps of the renal pelvic mucosa, a further indication that saccharin would be carcinogenic for the similar mucosa of the urinary bladder.

### Tumors Were Caused by Stones in the Urinary Bladder

There was never any evidence that the tumors were caused by stones in the urinary bladder. There was even less evidence in one study that "in the case of the third rat with bladder lesions, the reason is uncertain, for no stones were found though they might have been present and later voided" (6). If one carefully examines the data in the rats that did have both stones and tumors, the lesions in the urinary bladder preceded the occurrence of stones, and in no instance did the stones precede the development of tumors of the urinary bladder (17, 20). The relationship was best observed in the study in which not one rat with a tumor of the urinary bladder had stones (20). Conversely, not one lesion or tumor of the urinary bladder was found in the large number of rats with stones in the bladder.

### Tumors Were Caused by Parasites in the Urinary Bladder

Those searching for an explanation for the tumors in the urinary bladder in rats that did not have stones or that presumably "voided" the stones after a tumor developed came up with parasites. The tumors were not induced by saccharin, but they were caused by parasites in the urinary bladder. They cited a study "A nematode, . . . , the adult

form of which lives in the rat bladder has been found to increase significantly the number of bladder tumors developing in rats fed . . ." (25). The data of that study, tumors in 5 of 156 rats with parasites, did not demonstrate that parasites were responsible for the development of tumors of the bladder. The author did state that "because none of our rats developed calculi, it seems unlikely that the stone formation mechanism holds true in this case." Parasites also were not observed in the study in which the development of tumors of the urinary bladder was not associated with stones in the urinary bladder (20).

### **Tumors Were Not Dose-Related and Occurred Only at the Highest Doses**

Chronic renal disease results from the administration of low doses of saccharin and tumors of the renal pelvis and urinary bladder develop with the higher doses. Tumors or polyps of the renal pelvis in rats ingesting saccharin were clearly dose related; however, tumors of the urinary bladder were observed in rats given the highest dose levels. One female rat ingesting 0.05% saccharin developed an undifferentiated malignancy of the urinary bladder (19). At lower doses saccharin may not reach the urinary bladder and could not be expected to produce tumors. Tumors developed in organs other than the urinary bladder in animals given lower doses. Chronic renal disease in rats given the lower, as well as the higher doses, may have shortened the life span and/or interfered with the development of tumors. It is not necessary for tumors to develop at all dose levels in order for a chemical to be carcinogenic.

### **Tumors Were Caused by the Impurity, *o*-Toluenesulfonamide, But Not by Saccharin**

It was highly unlikely that the impurity, *o*-toluenesulfonamide, was inducing the tumors of the urinary bladder. The doses, compared to those of saccharin, were relatively small. The fact that human beings were consuming the same chemicals makes this criticism irrelevant. More recent studies used saccharin that had fewer impurities or none at all (14, 19, 24, 31), and animals developed tumors just as they did in earlier studies. In a recent study, *o*-toluenesulfonamide was not carcinogenic for the urinary bladder of rats (24).

It is worth noting that human beings ingesting saccharin also are in contact with other chemicals, and that in the past saccharin and cyclamate were

ingested together. (Some studies in which saccharin was given with cyclamate were regarded as irrelevant; however, for these reasons such studies should have been considered in the evaluation of saccharin.) It is particularly well put in one study: "Man, however, is exposed to a complex environment, in which he may simultaneously encounter potentially carcinogenic chemicals in the food he eats, in the air he breathes, in the tobacco smoke he inhales, and in the factory where he works" (18).

### **Tumors Developing in the Bladder Implantation Study and Certain Other Studies Were Irrelevant**

The carcinogenicity of saccharin was studied by implanting pellets of cholesterol and saccharin into the urinary bladder of mice. This route of administration, i.e., direct application to the urinary bladder, was considered as not appropriate, even though saccharin is concentrated in the urinary bladder of animals (41). Shimkin, at an NCI Subcommittee Meeting, recently pointed out that the severity of the lesions, as well as the route, needed to be taken into consideration (42). An FDA official also stated that all studies, including bladder implantation studies, should be considered in the evaluation of a chemical for carcinogenicity (43).

Tumors developed in the urinary bladder of mice with saccharin and cholesterol pellets (26, 27). These tumors in the urinary bladder of mice with saccharin implants were often undifferentiated and highly anaplastic. The tumors also were more malignant than those induced by cyclamate and were comparable to those induced in mice, rats, and dogs by other carcinogens. Saccharin also was cocarcinogenic for the urinary bladder in rats and was a tumor initiator when applied to the skin (17, 18, 35).

### **Tumors of the Urinary Bladder Were Not Preceded by the Development of Preneoplastic Lesions and Were Not Very Malignant**

The tumors of the urinary bladder in rats given saccharin in at least two studies were preceded by the development of preneoplastic lesions, i.e., hyperplasia (17, 20), and are similar to tumors developing in the urinary bladder of rats given other chemicals. Hyperplasia, observed as early as 4 months, was mild, moderate, or severe in degree. The hyperplasia later became polypoid and progressed to carcinomas.



Tumors in rats were diagnosed as carcinomas based upon morphologic criteria by the NAS Pathology Subcommittee, as well as others (16, 22). The carcinomas sometimes were invasive. Tumors of the urinary bladder in mice were often undifferentiated and highly anaplastic, invasive, and occasionally metastatic (26). They were more malignant than those induced by cyclamate and were comparable to those induced in mice, rats, and dogs by other carcinogens.

Tumors need not have metastasized in order to be malignant (44). Diagnoses of carcinomas can be based on morphologic criteria. Animals often do not live very long after the development of carcinomas. Therefore, it is not unusual to find carcinomas in rats that have not metastasized (45). In most animal studies, a careful search was not made for metastases. In order to be certain that metastases have not occurred, it would be necessary to do serial sections, not only of lymph nodes, but of other organs as well.

### Studies Were Not Well Planned

The plan of some studies was criticized by the NAS Committees (46, 47). In recent studies, these criticisms were taken into consideration. Treated rats were exposed to saccharin from inception (19, 24, 31). Control rats were given sodium carbonate (31), the saccharin contained fewer or no impurities (14, 24), and one study was carried out for 28 months (31).

### Tumors Did Not Develop in Organs Other than the Urinary Bladder

National Academy of Sciences Committees, as well as some investigators, felt that saccharin did not induce tumors in organs other than the urinary bladder; however, benign and malignant tumors, or malignant tumors alone were increased in male and female rats ingesting saccharin. Male rats were somewhat more susceptible to the development of malignant tumors than were female rats, more so at the highest doses. Unusual malignant tumors were sometimes found in saccharin-treated rats and not in the controls. There were also increased incidences of tumors at specific sites in mice and rats ingesting saccharin. Tumors were found chiefly in the reproductive and hematopoietic systems, and in the lungs, vascular system, and squamous epithelium. Therefore, organs other than urinary bladder and renal pelvis in rats and mice ingesting saccharin had benign and malignant tumors.

### Studies Have Shown Saccharin to be Safe for Humans

Saccharin is rapidly absorbed, distributed throughout the body, and excreted unchanged, except for 1% or less as metabolites, in the urine of animals (48-50). Saccharin is concentrated in the kidney and urinary bladder (41, 51). Saccharin crosses the placenta and is found in all tissues of the fetus of subhuman primates (48). Another nonnutritive sweetener, cyclamate, is present in largest amounts in the liver, spleen, and kidney of the fetus following placental transmission in human and macaque pregnancies (52).

Although reports of many studies were available, conclusive evidence was lacking as to whether or not saccharin is mutagenic. A study using more sensitive methodology and tester strains found that saccharin preparations commonly distributed as artificial sweeteners were mutagenic in bacterial tests (53). Highly purified saccharin was not mutagenic in the direct assay, but the urines of mice given this material were mutagenic.

Results from animal studies can be used to predict the impact of the test substance on human health (54).

"Qualitatively, it has been shown that the formation and occurrence of tumors in man and other mammals—such as hamsters, mice and rats—is quite similar. In tests undertaken to date, it had been demonstrated, with one possible exception (arsenic), that every chemical which has been found to be carcinogenic in man is also carcinogenic in one or more mammalian test animals. Furthermore, the majority of chemical carcinogens which have undergone sufficient testing have been shown not to be species specific. That is, testing has shown that if these compounds will produce tumors in one species, it will very likely produce tumors in more than one, thus adding weight to any finding of carcinogenicity in tests using any mammalian species. Sufficient documentation is available on qualitative extrapolation of animal data that one must conclude that a finding of carcinogenicity in one mammalian species should be deemed to have relevance for other mammalian species—including man."

"Quantitatively, one must assess the relationship between the dosage required to produce tumors in test animals and that required in man. This assessment, difficult as it is, is complicated by the fact that man is much more variable in his physical characteristics than are most other species of mammals. One can state, however, that it is quantitatively impossible to establish any safe level of distribution of a carcinogen in the environment. A safe level may exist for a particular human being, but because

there is such variation among human beings, it is impossible to establish such a level for a community. Furthermore, review of the literature on carcinogenicity tests suggests that no such null effect threshold or safe level has been determined in test animals—much less for man—for virtually any known carcinogen.”

The three epidemiological studies which are reported as showing no increase in cancer of the urinary bladder in humans, have been critically reviewed elsewhere (55–58). A recent study in Canada concluded that there is an estimated 60% increase in the risk of cancer of the urinary bladder associated with the use of artificial sweeteners (primarily sac-

charin) in men (59, 60). The risk ratios increased with both frequency and duration of saccharin usage, with the trend being statistically significant in both cases. For all men using saccharin 7%, and for diabetic men 33%, of cancer of the urinary bladder might be ascribed to the use of artificial sweeteners.

## Summary of the Carcinogenicity Studies in Animals

The findings of the carcinogenicity studies in rats, mice, and other animals are summarized in Tables 37, 38, and 39.

Table 37. Summary of carcinogenicity studies in rats.

Authors	Stock	Generation, age, sex	Concentration, %	Duration	Carcinogenicity	Comments
FDA (1948-1949)	Osborne-Mendel	F <sub>0</sub> (3 weeks) male, female	0,0.01,0.1 0.5,1,5	2 yr	Lymphosarcoma Renal pelvis	Urinary bladder not examined Chronic renal disease Hyperplasia bone marrow
Lessel	Boots-Wistar	F <sub>0</sub> male, female	0,0.005,0.05, 5	2 yr	Urinary bladder lesions	Some bladder stones
Litton Bionetics, Inc.	Charles River CD	F <sub>0</sub> male, female	0, 1, 5	2 yr	All sites Urinary bladder tumors	No bladder stones Chronic renal disease
Bio-Research Consultants	Charles River Sprague-Dawley	F <sub>0</sub> male, female	0, 1, 5	2 yr	Urinary bladder tumors	Hyperplasia renal pelvis No bladder stones Some parasites
Munroe, et al.	Charles River	F <sub>0</sub> weanling male, female	0,0.15,0.5,1.5,5	26 mo	Urinary bladder carcinoma	No bladder stones with tumors No parasites
Warf Institute	Sprague-Dawley	F <sub>1</sub> male, female	0,0.05,0.5,5	100 wk	Urinary bladder carcinoma All sites Uterus and ovary	No bladder stones, Parasites and renal disease not mentioned
FDA (1973)	Charles River Sprague-Dawley	F <sub>1</sub> male, female	0,0.01,0.1,1, 5, 7.5	14,18 mo	Urinary bladder carcinoma Renal pelvis Mammary gland All sites	No bladder stones with tumors No parasites Hyperplasia of urinary bladder Chronic renal disease
		F <sub>0</sub> male, female		4,6,12 mo		Hyperplasia of urinary bladder
Arnold, et al.	Charles River Sprague-Dawley	F <sub>0</sub> male, female F <sub>1</sub> male, female	0, 5	148 wk 127 wk	Urinary bladder tumors Urinary bladder carcinoma	No parasites No relationship of bladder stones to tumors Incidence higher in F <sub>1</sub> than F <sub>0</sub> Metastases from urinary bladder carcinoma to lymph node
Schmähli		F <sub>0</sub> male, female	0,0.2,0.5	Lifetime	Lymphoma and leukemia	High death rats from respiratory infections
NIHS, Tokyo	Sprague-Dawley	F <sub>0</sub> male	5	12,28 mo	Lung tumors Skin tumors	Data incomplete
Hicks et al.	Wistar	F <sub>0</sub> , 150 g, male, female	4, 8	95 wk	Urinary bladder tumors	Controls for cocarcinogenesis studies.

Table 38. Summary of carcinogenicity studies in mice.

Authors	Stock	Age, sex	Concentrations, %	Duration	Carcinogenicity	Comments
Bio-Research Consultants	Albino (HaM/ICR)	8 wk male, female	0, 1, 5	> 104 wk	All sites Lung Vascular Uterus	
NIHS, Tokyo	dde	Male, female	0, 0.2, 1, 5	12, 18, 21 mo	Ovary	Data incomplete
Roe et al.	Albino	Female	5	18 mo	Inconclusive	Controls given single dose of vehicle PEG for benzopyrene study Control and test mice were different ages at start of experiment
Kroes et al.	Swiss-SPF	Male, female 70-90 days	0, 0.2, 0.5	Long-term	Amyloidosis kidney Urinary bladder tumors	Swiss-Webster mice not suitable for such a study

Table 39. Summary of other carcinogenicity studies.

Authors	Stock species	Age, sex	Concentrations	Duration	Carcinogenicity	Comments
Hicks et al.	Wistar rats	Male, female	4, 8% oral	95 wk	Cocarcinogenic for urinary bladder carcinomas with MNV	
Bryan et al.	Swiss mice	Female 60-90 days	Cholesterol + saccharin pellets in urinary bladder (5-6 mg saccharin)	13 mo	Urinary bladder carcinomas	
Allen et al.	Mouse	stock	Cholesterol + saccharin pellets in urinary bladder (approximately 3 mg saccharin)		Urinary bladder tumors	
Althoff et al.	Syrian golden hamsters	Male, female	0, 0.156, 0.312, 0.625, 1.25%	62 wk	Inconclusive	Short duration
Golberg Short duration	Rhesus	Male, female	0, 20, 100, 500 mg/kg-day	79 mo	Inconclusive	Data incomplete

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